UNIVERSITAT DE BARCELONA

Contaminants organoclorats, organobromats i organofosforats en població general

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CONTAMINANTS ORGANOCLORATS, ORGANOBROMATS I ORGANOFOSFORATS EN POBLACIÓ GENERAL

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Programa de Doctorat:

Química Analítica i Medi Ambient

Memòria presentada per optar al

Títol de Doctor per la Universitat de Barcelona

CONTAMINANTS ORGANOCLORATS, ORGANOBROMATS I ORGANOFOSFORATS EN POBLACIÓ GENERAL

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Barcelona, Juliol de 2019

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«Se puede estar realmente convencido de querer algo -quizá durante años-, si se sabe que el deseo es irrealizable. Pero si de pronto se encuentra uno ante la posibilidad de que ese deseo ideal se convierta en realidad, solo se desea una cosa: no haberlo deseado.»

Michael Ende, *La historia interminable*, 1979

Agraïments

En primer lloc, agreixo al Joan, el meu director de tesi, oferir-me l'oportunitat de quedar-me al grup i realitzar una tesi doctoral. Fer el doctorat mai havia estat una opció per a mi però m'ha servit de molt i, sobretot, he après i crescut molt a nivell personal i laboral. També m'agradaria agrair als altres investigadors del grup, la Pilar, el Barend, el Jordi López i la Belén i el meu tutor a la UB, Xavier Santos, que m'han ajudat sempre que ho he necessitat.

A los compañeros de grupo que me han acompañado durante todos estos años o parte de ellos, Óscar, Marta Casado, Yolanda, Bibi, David, Marta Fontal, Anton, Lourdes, Anuar, Patricia y Esther. A los actuales compañeros de grupo, Rai, Nupur, Marco y los que nos visitan durante unos pocos meses, que a veces saben a poco, Soraya, Sergi, Alex, Aina, Núria, Ariadna y Sergi. Por último, las chicas de salud, Esther, Mercè y Marta que me acogieron como una amiga más cuando llegué y Eva con la que he vivido esta experiencia casi en paralelo. Como siempre hemos dicho: "Con las horas que pasamos juntas suerte que nos llevamos bien!".

A todo el personal del centro però en especial a las tecnicas de las salas de gases i masas (Inma, Roser y Dori) que se encargan de que todos los equipos estén listos.

Un agradecimiento muy especial a todos mis Pelicanos (y los que no quieren serlo) con los que he compartido muchos momentos a lo largo de estos años,

algunos nos conocemos casi desde el primer dia que pisamos la facultat de química y ahí seguimos... espero que sea así por muchos años mas.

Gracias a mi familia por su respaldo y su ayuda en todo momento y como no a los pequeños de la casa que me entretienen y distraen cada vez que me ven "Tita, vamos a jugar!".

Finalmente, muchas gracias a Guillem por todo, por estar conmigo siempre, quererme y apoyarme en todo lo que hago. Porque después de tantos años ya no recuerdo como era estar sin él. *Un petonet!*

Índex

Resum

Durant les dècades dels anys 50 i 60 del segle XX es van utilitzar arreu del món diferents tipus de compostos organoclorats i organobromats, que pertanyen als contaminants orgànics persistents (COPs), com a pesticides, retardants de flama o aïllants tèrmics. El 2001 la Convenció d'Estocolm sobre COPs va reconèixer els efectes adversos d'aquestes compostos sobre la salut i el medi ambient i va proposar controls amb l'objectiu d'eliminar o reduir el seu ús. Com a conseqüència d'aquesta prohibició altres compostos s'han anat sintetitzat per substituir-los, alguns d'ells són els pesticides organofosforats i els piretroides, que tot i que no són persistents el fet que s'utilitzin en grans quantitats fa que siguin un risc pel medi ambient i la salut.

Aquest estudi avalua, per una banda, l'abast de diferents contaminants orgànics persistents (COPs), especialment els compostos organoclorats (OCs) i els polibromodifenil èters (PBDEs), en dones. I per altra banda, la càrrega i exposició de diferents pesticides organofosforats i piretroides en població general de tres països europeus, dos països on l'agricultura esta molt present per tant s'utilitzen molts pesticides, Espanya i Itàlia, i un en el que es consumeixen menys, Eslovènia.

La recerca sobre COPs es centra en dues poblacions de l'Argentina (Salta i Ushuaia) i una de Rússia (Chukotka), Ushuaia i Chukotka es poden considerar remotes. S'ha realitzat una anàlisi química d'un ampli ventall de COPs en mostres de sèrum de dones embarassades, emprant cromatografia de gasos i

espectrometria de masses. A més, les concentracions de COPs han estan contrastades amb una sèrie de factors sociodemogràfics. El resultat més destacable es que totes les dones en totes tres poblacions tenen nivells detectables d'algun COP tot i que es van prohibir fa anys. A més, tant la residència de cada una d'elles com l'edat, paritat i l'IMC son uns factors que influencien les concentracions d'OCs. També s'ha trobat una associació positiva entre les concentracions de 4,4'-DDT amb l'edat gestacional, el pes i la longitud dels nadons, suggerint una interacció a nivell metabòlic entre aquest compost i el fetus.

L'estudi sobre pesticides organofosforats i piretroides determina la exposició de diferents tipus de poblacions, nens de Trieste (Itàlia), parelles de dones i els seus nens de Ljubljana (Eslòvenia), dones embarassades de Tarragona (Espanya) i població general de zones rurals de Carbia i Sucs a Galícia i Catalunya, respectivament. S'ha desenvolupat una metodologia analítica que permetés les anàlisis dels diferents metabòlits dels pesticides organofosforats i piretroides en l'orina dels grups esmentats. S'ha vist que tant els treballadors agrícoles de Sucs, com les persones que viuen a un entorn rural (Sucs i Carbia) estan més exposats a pesticides organofosforats i piretroides. A més, s'ha trobat una distribució diferent en quant als pesticides organofosforats respecte a altres estudis, el pirimifos té un pes molt important en la població de dones embarassades de Tarragona i nens d'Itàlia. Finalment, s'ha avaluat l'exposició a pesticides organofosforats i piretroides i la seva relació amb la manipulació i utilització d'equips de protecció individual en la població agrícola de Sucs, concloent que l'ús d'EPI es útil i necessari en aquests tipus de treballs.

Abreviacions

3-PBA	Àcid 3-fenoxibenzoic
4-F-3-PBA	Àcid 4-fluor-3-fenoxibenzoic
AMAP	*Arctic Monitoring and Assessment Programme*
ATSDR	Agència per a Substàncies Tòxiques i el Registre de Malalties
BDE	Bromodifenil èter
CIL	*Cambridge Isotope Laboratories*
CMHC	3-cloro-4-metil-7-hidroxicoumarin
COP	Contaminant orgànic persistent
DDD	Diclorodifenildicloroetà
DDE	Diclorodifenildicloroetilè
DDT	Diclorodifenitricloroetà
DEAMPY	2-dietilamino-6-metilpirimidin-4-ol
EC	*European Commission*
EEUU	Estats Units
EMASAR	Estudio del Medio Ambiente y la Salud Reproductiva
EPA	*Environmental Protection Agency*
EPI	Equip de Protecció Individual

GC-ECD	Cromatògraf de gasos amb detector de captura d'electrons
GC-NICI MS	Cromatògraf de gasos amb espectrometre de mases en ionització química negativa
G-EQUAS	*The German External Quality Assessment Scheme*
GMP	*Global monitoring plan*
HCB	Hexaclorobenzè
HCH	Hexaclorociclohexà
HDL	Lipoproteïnes d'alta densitat
HEALS	*Health and Environment-wide Associations based on Large population Surveys*
HELIX	*The Human Early-life Exposome*
IARC	Agència Internacional d'Investigació del càncer
IMC	Índex de massa corporal
IMPY	2-isopropil-6-metil-4pirimidol
LD	Límit de detecció
LQ	Límit de quantificació
LDL	Lipoproteïnes de baixa densitat
MDA	Àcid dicarboxílic del malatió
MG	Mitjana geomètrica
nd	No detectat
nq	No quantificat
OC	Compostos organoclorats
OCP	*Organochlorine pollutants*

OMS	Organització Mundial per la Salut
OP	Organofosforat
ONU	Organització de les Nacions Unides
PBDEs	Polibromodifenil èters
PCB	Policlorobifenil
PeCB	Pentaclorobenzè
PNP	4-nitrofenol
POP	*Persistent organic pollutants*
PTS	*Persistent toxic substances*
PYR	Piretroide
TBB	1,2,4,5-tetrabromobenzè
TCPY	3,5,6-tricloro-2-piridinol
UE	Unió Europea
UNEP	Programa de les Nacions Unides per al Medi Ambient

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CAPÍTOL 1. INTRODUCCIÓ

Capítol 1: Introducció

La química ambiental estudia els processos químics o bioquímics que tenen lloc en els sòls, rius, llacs, oceans, atmosfera, i els intercanvis entre aquests compartiments. Això permet conèixer l'impacte que les activitats humanes provoquen al nostre entorn i la problemàtica que això comporta. Un aspecte d'aquesta disciplina és la toxicologia ambiental, que estudia els efectes tòxics de les molècules que es troben en els ecosistemes sobre els organismes que hi viuen o sobre l'ecosistema mateix.

Avui dia, és evident que l'activitat humana té una gran influència sobre el funcionament de molts aspectes importants del nostre planeta i és previsible que l'impacte d'aquesta activitat augmenti. Bona part de la interacció humana sobre el planeta terra i els seus ecosistemes és deguda a l'emissió de compostos químics en quantitats superiors a les que poden eliminar. L'impacte humà ha causat pèrdues de biodiversitat, canvis en el paisatge, canvis en el clima o efectes en la salut de les persones.

Els humans són part dels ecosistemes. Una persona pot estar algunes setmanes sense menjar, alguns dies sense beure i només alguns minuts (pocs) sense respirar. Aquests elements imprescindibles per la vida humana provenen dels ecosistemes. Fins i tot l'oxigen de l'aire és un subproducte de la fotosíntesi que no es trobava a la terra al seu inici, quan era un planeta biològicament inert. Lamentablement, l'acció humana ha introduït en el medi ambient diversos compostos que són nocius per la salut humana. Alguns d'aquests són químicament molt estables i un cop introduïts no es degraden. Altres més

degradables sovint s'introdueixen en el medi en quantitats i fluxos tan alts que fan que s'hi trobin habitualment. Tant en un cas com l'altre, els humans es troben exposats a aquests compostos durant períodes molt llargs de la seva vida.

Recentment s'ha definit l'exposoma com a l'estudi integral de tots els compostos químics que poden influir en la salut humana al llarg de la seva vida.

1.1 Exposoma

El terme exposoma va proposar el 2005 pel epidemiòleg Christopher Wild en un article titulat *Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology*. Aquest concepte és va proposar per dirigir l'atenció sobre la necessitat de millorar i completar el coneixement dels compostos presents en el medi ambient que afecten a la salut humana (Wild, 2005).

L'exposoma és l'estudi integral de les exposicions medi ambientals a compostos tòxics que experimenta una persona al llarg de la seva vida. Pretén identificar, caracteritzar i quantificar les exposicions exògenes i endògenes, així com determinar els factors de riscs i possibilitar la predicció de futures malalties que pot patir una persona durant la seva vida com a conseqüència de l'exposició a contaminants (HEALS, 2013). La procedència de les diferents exposicions es classifiquen en tres dominis: intern, extern específic i extern general (Wild, 2012).

El domini de les exposicions generals externes inclou els aspectes socials, econòmics i influències psicològiques de cada individu, com ara: capital social, educació, estatus econòmic, estrès psicològic i mental, ambient urbà o rural i el clima. Les exposicions específiques externes inclouen agents infecciosos, contaminants químics, dieta, factors de l'estil de vida (alcohol, tabac,...), ocupació o intervencions mèdiques. El domini intern comporta els processos

biològics que tenen lloc en el cos de cada individu com a resposta a les exposicions externes (Wild, 2012).

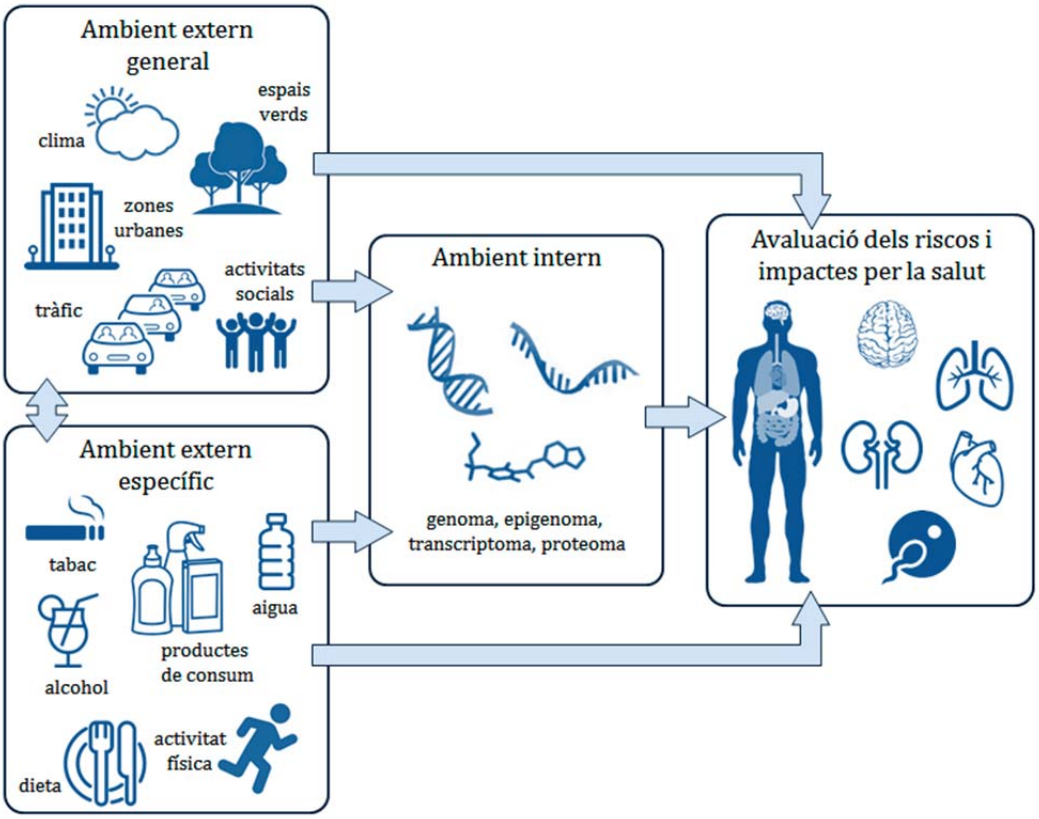

Figura 1.1 Efectes de la interacció entre els diferents dominis que constitueixen l'exposoma (ambient extern general i específic i ambient intern) i els riscos per la salut (Vrijheid, 2014).

El concepte original de Wild va ser ampliat més tard per Rappaport i Smith (Rappaport i Smith, 2010), que van definir l'exposoma en termes de compostos químics circulant pel cos com a conseqüència tant de les exposicions endògenes com exògenes. En altres paraules, l'exposoma representa les exposicions combinades provinents de diferents fonts que arriben a l'ambient químic intern del cos.

Així doncs, mesurar l'exposoma en matrius humanes com sang o orina ofereix una aproximació interesant per estudiar els processos d'exposició-

associació, ja que la sang transporta els compostos químics des de i fins els teixits i representa un dipòsit dels compostos endògens i exògens en el cos en un moment determinat. L'orina, en canvi, és una de les vies d'excreció dels compostos que no interessa conservar en el cos (Rappaport et al., 2014).

La naturalesa dinàmica de l'exposoma fa que la seva caracterització sigui un aspecte molt exigent. Per estudiar-lo de manera total en un individu es necessiten mesures múltiples al llarg de la vida. També és important tenir en compte l'ambient particular on es desenvolupa una persona en les primeres etapes de la vida. Per tant, aquestes mesures han d'incloure les exposicions prenatals (Wild, 2012). Un dels punts clau per a la caracterització de l'exposoma és la biomonitorització de poblacions humanes, que proporcionin dades estadístiques de la interacció entre contaminants ambientals, acumulació en el domini intern humà i els efectes sobre la salut.

En aquesta memòria de tesi s'han estudiat alguns dels contaminants orgànics que més poden afectar a la salut humana en dos matrius diferents, sèrum sanguini i orina, centrant-nos en l'avaluació dels riscos i alguns dels impactes, que comporta la presència d'aquests compostos, sobre la salut. Per una banda en sèrum sanguini s'han analitzat contaminants que són persistents, compostos organoclorats (OCs) i organobromats. Per l'altra banda, en orina s'han analitzat els compostos polars no persistents, concretament pesticides organofosforats (OPs) i piretroides (PYRs), que el metabolisme humà pot transformar i excretar.

1.2 Compostos organoclorats, organobromats, pesticides organofosforats i piretroides

Durant les dècades dels anys 50 i 60 del segle XX es van utilitzar arreu del món diferents tipus de compostos organoclorats i organobromats, que pertanyen als coneguts contaminants orgànics persistents (COPs), com a pesticides, retardants de flama o aïllants tèrmics. Amb el pas del temps, es van començar a publicar estudis on es relacionaven alguns d'ells amb problemes mediambientals i de salut. Entendre i abordar aquests impactes no ha estat un camí fàcil i, a més de la recerca científica, en algunes ocasions ha anat lligat amb l'activisme social.

El progrés per entendre els efectes perjudicials d'alguns COPs va començar en el 1962, quan la biòloga Rachel Carson, dels EEUU, va publicar el llibre *Silent Spring*, que va provocar una sensibilització sobre l'impacte dels pesticides en el medi ambient. El llibre suggeria que el DDT i altres pesticides podien tenir efectes adversos en la vida salvatge, en particular les aus (Carson, 1962). Encara que el seu treball va ser molt controvertit en aquella època va impulsar el naixement de moviments mediambientals i va provocar canvis importants als EEUU, com ara la prohibició del DDT i altres pesticides i la creació de l'Agència de Protecció Mediambiental, *Environmental Protection Agency* (EPA, 2018).

Com a conseqüència d'aquests esdeveniments, la mateixa dinàmica es va estendre a altres països. El 2001 l'Organització de les Nacions Unides (ONU) va elaborar un tractat sobre el tema, la Convenció d'Estocolm sobre contaminants orgànics persistents, que va reconèixer els efectes adversos tant pel medi ambient com per la salut d'aquests compostos i va proposar controls amb l'objectiu d'eliminar o reduir la producció i ús de dotze COPs seleccionats (OMS i UNEP, 2001). Al llarg del temps s'han anat incloent altres COPs a la llista de composots prohibits o restringits (OMS i UNEP, 2009b).

Com a resultat de la prohibició dels COPs abans esmentats altres tipus de compostos es van començar a sintetitzar per ser comercialitzats, tot donant lloc a compostos orgànics que tot i que no són persistents, com els abans citats, la seva utilització en grans quantitats fa que estiguin presents usualment en el medi ambient.

El sector agrícola va ser un dels grups que va notar més la prohibició de pesticides reglats com a contaminats orgànics persistents. Com a conseqüència, la indústria química no ha deixat de desenvolupar pesticides sintètics nous pel seu ús en agricultura o ramaderia. Entre aquests hi ha els pesticides organofosforats i els piretroides. Com s'ha esmentat abans la no persistència d'aquests compostos no els fa innocus, i també tenen un impacte sobre el medi ambient i la salut.

De la mateixa manera que es va crear el Conveni d'Estocolm per regular els COPs, en resposta al creixement espectacular de la producció i el comerç de productes químics (OMS et al., 2004), el 2004 va entrar en vigor el Conveni de Rotterdam sobre el procediment de consentiment fonamentat previ aplicable a certs plaguicides i productes químics perillosos objecte de comerç internacional. En aquest segon conveni hi ha tant els pesticides persistents inclosos en el Conveni d'Estocolm com alguns altres que es van començar a utilitzar més tard, com per exemple el paratió.

La present tesi doctoral examina les concentracions de diversos compostos organoclorats, organobromats i organofosforats, que s'han produït per ser utilitzats en l'agricultura o la industria, en poblacions representatives d'Argentina, Rússia, Itàlia i Eslovènia. Amb l'objectiu d'augmentar el coneixement científic sobre la distribució i repercussió d'aquests contaminants en la salut humana.

1.3 Biomonitorització en poblacions humanes

Es coneix com biomonitorització la utilització d'organismes vius per avaluar la importància de la contaminació ambiental. Òbviament, es troben entre aquests els éssers vius i en aquest cas la càrrega de compostos químics o els seus metabòlits tòxics en matrius biològiques (com sang, orina o llet materna) són objectes d'estudi. Sovint, els estudis de biomonitorització es centren en l'anàlisi de contaminants en poblacions especialment vulnerables a aquests tipus de compostos, com ara nens o dones embarassades. També es poden trobar estudis en persones que estan particularment exposades ja sigui laboralment o per viure en zones contaminades (per exemple, els estudis fets a habitants i treballadors de la fabrica clor-alcalí de Flix, Catalunya)

El Pla Global de Monitorització (GMP) del Conveni d'Estocolm recomana la recol·lecció de dades comparables de totes les regions del món per avaluar la seva efectivitat en minimitzar l'exposició humana i mediambiental a COPs (OMS i UNEP, 2009a). L'objectiu d'aquest pla és identificar tendències temporals i espacials dels nivells de COPs en humans (saber, per exemple, si les concentracions augmenten o disminueixen amb el temps).

A part del GMP, que funciona a nivell internacional, existeixen altres entitats que fan seguiment a diferents grups de la població a nivell nacional, que a més dels COPs estudien altres tipus de compostos incloent els pesticides organofosforats i piretroides. Tant el *National Health and nutrition Examination Survey* (NHANES, 2017), un programa d'estudis designat per avaluar la salut i estatus nutricional d'adults i nens als EEUU, com el *German Environmental Survey* (GerES, 2017) són bons exemples de programes de monitorització.

En els últims anys han sorgit diversos programes de biomonitorització aplicats a l'exposoma. Alguns d'aquests projectes són el *Health and*

Environment-wide Associations based on Large population Surveys (HEALS, 2013) i *The Human Early-life Exposome* (HELIX, 2018).

Tot i això, encara es poden trobar molts països on la informació sobre la concentració de contaminants en la població és molt limitada, o no existeixen estudis amb una mostra representativa. Una de les tasques dels científics que treballem en aquest camp és el de contribuir a omplir els buits d'informació que queden.

1.4 Epidemiologia ambiental

L'epidemiologia és l'estudi i anàlisi de la distribució, la freqüència, les causes i el control dels factors relacionats amb la salut i les malalties en poblacions humanes, amb l'objectiu de millorar la salut de la població. En epidemiologia s'estudia la salut dels grups en relació el seu medi (físic, cultural o econòmic). L'epidemiologia ambiental és una de les branques d'aquesta disciplina que es dedica a determinar com és l'impacte de l'exposició a contaminació ambiental sobre la salut humana (Pearce i Douwes, 2017).

Els estudis epidemiològics es poden dividir en dos grans grups. Per una banda els estudis observacionals, on els investigadors observen l'exposició dels voluntaris sense intervenir i després analitzen els diferents patrons segons les diferències entre els individus i per altra banda, els estudis experimentals, on hi ha un disseny previ que vol conèixer com afecta una exposició determinada a un compost en la població. Un exemple molt comú d'aquests tipus d'experiments és el de dividir un grup de persones en dos grups i exposar a un d'ells a un fàrmac i l'altre a placebo per després analitzar els efectes en cadascun dels grups.

Òbviament, en epidemiologia ambiental es realitzen estudis observacionals. Aquests, segons la informació disponible i el moment en que es realitzin, es poden dividir en estudis transversals (es desenvolupen en un moment concret

del temps) o longitudinals (en un període definit de temps). Dins dels estudis longitudinals alguns dels tipus d'estudis que s'empren més habitualment són els estudis de casos i controls o de cohort.

En aquesta tesi tots els estudis realitzats són de cohort, a continuació es detallen alguns dels trets més característics d'aquests tipus d'estudis.

Estudis de cohorts

En un estudi de cohort el grup de persones a estudiar comparteix una característica definitòria, aquesta pot ser experimentar un esdeveniment comú en un període seleccionat, com néixer en un determinat lloc o haver tingut un fill en un determinat moment.

El disseny epidemiològic de cohorts és el més adequat per l'estudi de malalties en períodes prolongats de temps. Clàssicament, un estudi de cohort pot ser classificat como a prospectiu, quan l'exposició pot haver tingut lloc abans de començar l'estudi i l'efecte no ha passat, o retrospectiu, quan tant l'exposició com l'efecte van tenir lloc abans de l'inici de l'estudi.

Un dels avantatges dels estudis de cohorts sobre altres dissenys d'investigació és la capacitat de proporcionar una mesura més adequada de l'exposició individual als objectes d'estudi, en aquest cas agents contaminants. Per aquest fi s'utilitzen diferents procediments, des de l'ús de qüestionaris fins l'ús de biomarcadors d'exposició. Si l'estudi és prospectiu, es redueixen al mínim els biaixos en la identificació de l'exposició. A més, es poden examinar efectes múltiples, aclarir la relació temporal entre exposició i efecte, determinar de manera directa la incidència de l'efecte en el grup d'exposats i no exposats i, si hi ha un seguiment de temps suficient, es poden arribar a estudiar diverses generacions.

No obstant això, els estudis de cohorts també presenten inconvenients: requereix d'una mida de mostra gran, poden ser costosos si s'ha de planificar

seguiments a llarg termini (prospectius) o es requereixen registres adequats (retrospectius), a més, la validesa dels resultats es pot veure afectada per l'abandonament de voluntaris al llarg del temps.

CAPÍTOL 2. OBJECTIUS I ESTRUCTURA

Capítol 2: Objectius i Estructura

2.1 Objectius

Avui dia la presència de contaminació tant en zones urbanes com rurals és un problema d'interès social notable perquè representa un risc per la salut humana i el medi ambient. Tot i que en les últimes dècades s'ha progressat molt en relació a la legislació en contra de la contaminació, el fet que existeixin lleis per controlar alguns contaminants no vol dir que el problema estigui solucionat. Molts d'aquests contaminants, un cop alliberats resten inalterats durant llargs períodes de temps al medi ambient.

En aquest context, aquesta tesi pretén avaluar per una banda els patrons d'acumulació dels contaminants orgànics persistents (COPs) més comuns, que estan prohibits des de l'any 2001, i per altra els pesticides organofosforats i piretroides més utilitzats en les últimes dècades, alguns d'ells prohibits i d'altres en ús. Per tant, els objectius proposats són:

- Examinar els nivells i patrons d'acumulació de determinats COPs en poblacions remotes (Argentina i Chukotka) segons diferents factors sociodemogràfics.
- Comparar els nivells de COPs entre les poblacions dels nostres estudis i altres poblacions d'arreu del món.
- Estudiar com les dependències entre residència i concentracions de COPs en sèrum de dones embarassades de Chukotka.

- Avaluar els efectes que pot tenir una exposició crònica en una població de dones embarassades presumptament exposada a COPs sobre els seus nens acabats de néixer.
- Desenvolupar un mètode per a l'anàlisi de pesticides organofosforats i piretroides en orina tant de població general com de població presumptament molt exposada.
- Estudiar els nivells i patrons d'acumulació dels metabòlits de pesticides organofosforats i piretroides en orina de diferents poblacions europees (Espanya, Itàlia i Eslovènia) en relació a diferents factors sociodemogràfics.

2.2 Estructura

La present memòria s'ha escrit com un compendi d'articles i s'ha estructurat en cinc capítols.

Capítol 1. Introducció: Es defineix la química ambiental i l'exposoma. Es presenten els compostos orgànics persistents i no persistents d'interès per la salut humana que són objecte d'estudi en aquesta memòria de tesi doctoral.

Capítol 2. Objectius i estructura: Es determina el marc de treball d'aquesta tesi.

Capítol 3. Contaminants orgànics persistents en zones remotes: Es presenten els treballs realitzats sobre contaminants orgànics persistents en mostres de dones embarassades de dues poblacions a l'Argentina i una a Rússia. Dues d'aquestes zones, Ushuaia i Chukotka, són considerades remotes. A més, s'han estudiat quins són els factors de risc en aquests tipus de poblacions.

Capítol 4. Pesticides organofosforats i piretroides en orina: Es presenta el desenvolupament i validació d'un mètode d'HPLC-MS/MS adequat per a l'anàlisi de metabòlits de pesticides organofosforats i piretroides en orina i

s'estudia la presència d'aquests compostos en diferents poblacions europees, població general i dones embarassades d'Espanya, nens d'Itàlia i parelles de nens i mares d'Eslovènia.

Capítol 5. Conclusions: Es resumeixen les conclusions generals a les que s'ha arribat a partir del treball realitzat durant aquesta tesi.

CAPÍTOL 3. CONTAMINANTS ORGÀNICS PERSISTENTS

Capítol 3: Contaminants orgànics persistents

3.1 Introducció

Els contaminants orgànics persistents (COPs) tenen origen antropogènic i es van produir bàsicament per ser utilitzats en l'agricultura i la indústria. Tenen en comú que la seva estructura és molt resistent a les transformacions químiques i bioquímiques del medi, són semi-volàtils, lipofílics i, el més important, són tòxics.

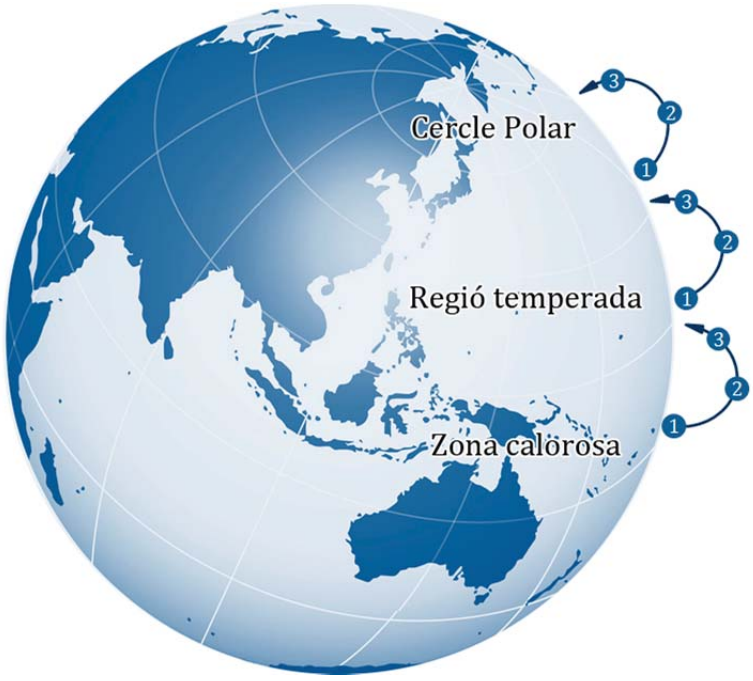

Figura 3.1 Transport de compostos des dels tròpics fins al cercle Polar a través de l'atmosfera. 1: Evaporació, 2: Transport, 3: Condensació.

Els COPs són altament persistents al medi ambient degut a la seva resistència a la degradació química, física i biològica. Per tant, es mantenen intactes durant llargs períodes de temps.

La seva volatilitat afavoreix la seva distribució més enllà del lloc d'utilització. Es poden transportar a nivell global a través de l'atmosfera, evaporant-se en llocs càlids per després condensar en zones més fredes. A aquest procés se l'anomena destil·lació global (Figura 3.1), i ha permès que estiguin àmpliament distribuïts pel món (Simonich i Hites, 1995), incloent-hi regions on mai s'han produït o utilitzat, com el cercle Polar, l'hemisferi sud o zones d'alta muntanya (Wania i Mackay, 1993; Grimalt et al., 2001; Arellano et al., 2014; Bravo et al., 2017).

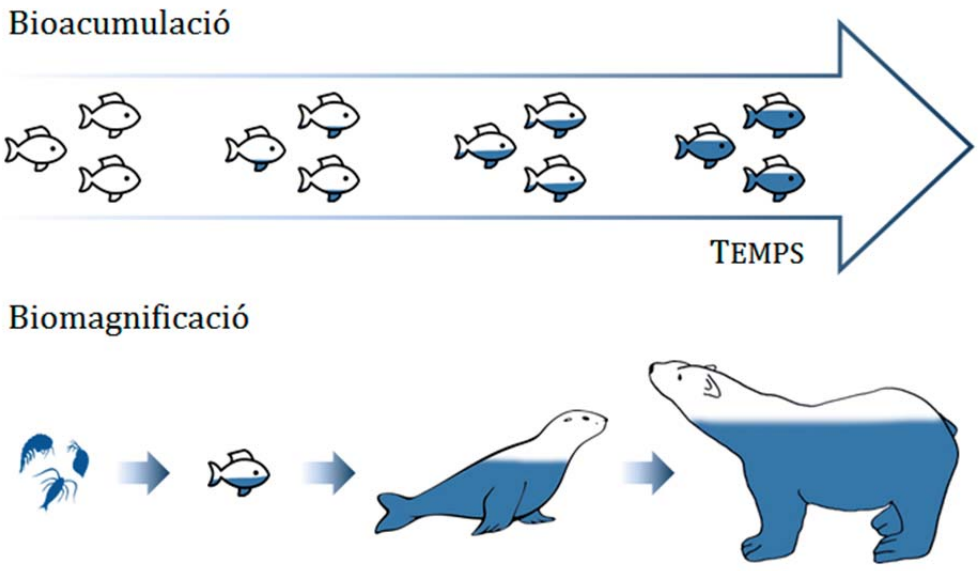

Figura 3.2 Esquema comparatiu entre bioacumulació i biomagnificació. En blau nivell de contaminació.

Degut a la seva lipofilicitat es bioacumulen fàcilment en els teixits grassos dels organismes vius i es biomagnifiquen a través de les cadenes tròfiques, sent una amenaça tant per a la vida salvatge com per a la humanitat (Figura 3.2).

Finalment, els COPs són potencialment tòxics. L'exposició humana a COPs pot arribar a comportar greus problemes de salut com ara càncer, disfuncions en els sistemes reproductiu i cardiovascular, o fins i tot defectes en les mesures antropomètriques dels nadons al néixer (Lerro et al., 2018; Wahlang, 2018; Bravo et al., 2019; Cabrera-Rodriguez et al., 2019).

L'any 1995 donada l'alta distribució d'aquest tipus de compostos i la seva toxicitat, l'ONU va donar un pas en contra dels COPs i va iniciar eles treballs que donaren lloc al Conveni d'Estocolm (signat el 2001 i implementat el 2004). Tot i haver-se deixat d'utilitzar i produir, un emmagatzematge inadequat o la seva presència en abocadors o compartiments ambientals fa que encara ara existeixin fonts potencials per a la incorporació d'aquests compostos en humans.

Els COPs estudiats en aquesta tesi són representatius de diferents orígens. En la pròxima secció es descriuen tots els contaminants orgànics persistents avaluats en aquesta memòria de tesi doctoral, que inclouen compostos organoclorats i organobromats.

Els compostos organoclorats

Els compostos organoclorats inclouen rang molt ample de compostos químics amb diferents estructures que foren sintetitzats per propòsits diferents. Aquest estudi s'ha centrat en dos dels grups més importants.

- Pesticides i els seus productes de degradació, com el pentaclorobenzè (PeCB), l'hexaclorobenzè (HCB), els diferents isòmers de l'hexaclorociclohexà (HCH) i el DDT i els seus metabòlits. Alguns d'aquests compostos, a més són subproductes en la síntesi industrial d'altres productes.
- Compostos que s'han produït sintèticament per les seves propietats aïllants i dielèctriques, com els policlorobifenils (PCBs).

Els dos grups esmentats tenen propietats fisicoquímiques similars, tot i que els seus usos i orígens són diferents. El nombre d'àtoms de clor i la seva posició en la molècula determinen el seu grau de persistència i toxicitat. Com més clors més insolubles són a l'aigua i més solubles en lípids, així com més resistents a la degradació.

A continuació es detallen tots els compostos organoclorats analitzats en aquest treball.

El pentaclorobenzè i l'hexaclorobenzè

El pentaclorobenzè i l'hexaclorobenzè pertanyen al grup dels clorobenzens. Estan formats per un anell de benzè substituït amb cinc i sis àtoms de clor, respectivament (Figura 3.3).

Abans de la seva prohibició, el PeCB s'utilitzava com a fungicida, com agent retardant de flama, en acceleradors de tints i en combinació amb policlorobifenils en fluids dielèctrics. També es feia servir com a intermedi en la síntesi d'alguns pesticides, com el pentacloronitrobenzè al qual actualment s'hi accedeix mitjançant una altra ruta sintètica per tal d'evitar el PeCB. Aquest compost també arriba indirectament al medi ambient com a resultat de la incineració de brossa, entre d'altres processos.

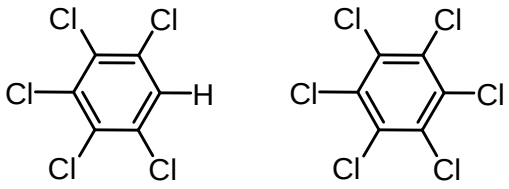

Figura 3.3 Estructura química del pentaclorobenzè (esquerra) i l'hexaclorobenzè (dreta).

La producció de PeCB es va aturar fa algunes dècades. Existeixen alternatives a aquest compost que són eficients i menys contaminants. Es va afegir al Conveni d'Estocolm l'any 2009, a l'annex A, per eliminar tant la

producció com el seu ús i a l'annex C, per reduir les emissions involuntàries (OMS i UNEP, 2009b).

L'HCB és un fungicida utilitzat per primera vegada l'any 1945 per tractar llavors de cultius agrícoles, sobretot de cereal, així com en la fabricació de focs artificials o municions. A l'actualitat és un subproducte de la producció de compostos clorats, dissolvents i diversos plaguicides. L'HCB és químicament molt estable i resistent a la degradació.

La producció industrial de l'HCB va començar a principis de 1930 i es va aturar durant la dècada dels 70 en la majoria de països occidentals. Ara per ara aquest compost està prohibit però es continua alliberant al medi ambient com a subproducte. Es va incloure al Conveni d'Estocolm l'any 2001 als annexos A, per eliminar la seva producció i ús, i annex C (per reduir les emissions involuntàries) (OMS i UNEP, 2009b).

Els seus efectes sobre la salut es van veure per primera vegada entre els anys 1954 i 1959 al Kurdistan, Turquia. Degut a l'escassetat d'aliments la població va utilitzar cereals tractats amb HCB per a la seva alimentació. L'enverinament massiu pel consum de llavors tractades amb HCB va provocar un gran número de morts, així com diversos símptomes com lesions a la pell, hiperpigmentació, hirsutisme, còlics i feblesa severa. Milers de persones van desenvolupar un trastorn metabòlic del fetge anomenat porfíria. Les dones embarassades que van ingerir llavors contaminades van passar l'HCB als fetus a través de la placenta i més tard a través de la llet, provocant la mort del 95% dels infants (Peters, 1976). Alguns estudis han demostrat recentment que una exposició moderada a l'HCB té efectes negatius en la reproducció (Eggesbo et al., 2009; Robledo et al., 2015). A més, una exposició crònica està relacionada amb diversos tipus de càncer (Grimalt et al., 1994; Waliszewski et al., 2005; Ploteau et al., 2017).

L'hexaclorociclohexà

L'hexaclorociclohexà té formula molecular $C_6H_6Cl_6$ amb un àtom de clor en cada carboni. Els àtoms de clor poden prendre diferents posicions relatives (cis/trans) donant lloc a 8 estereoisòmers amb diferents propietats fisicoquímiques (Figura 3.4).

D'entre tots els isòmers, l'únic actiu com a insecticida és el lindà (γ-HCH). Malgrat això es van comercialitzar barreges tècniques de diversos d'ells. La barreja tècnica d'HCH consisteix, aproximadament, en 60-70% α-HCH, 5-12% β-HCH, 10-15% γ-HCH, 6-10% δ-HCH i 3-4% ε-HCH (Kutz et al., 1991).

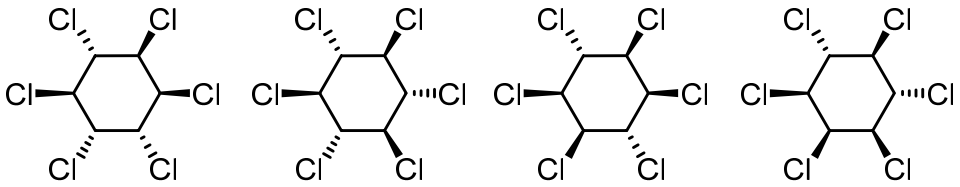

Figura 3.4 Estructura química de quatre isòmers del hexaclorociclohexà, d'esquerra a dreta: α-HCH, β-HCH, γ-HCH i δ-HCH.

Els HCHs es van sintetitzar per primer cop el 1825, però les seves propietats insecticides no es van descobrir fins el 1942 (Willett et al., 1998). En aquesta aplicació es van utilitzar, sobretot, en el tractament de sòls, llavors, fulles, arbres i fusta, així com a agents antiparasitaris en productes farmacèutics i veterinaris.

El lindà (γ-HCH) era l'ingredient actiu de molts sabons i xampús per al tractament de polls i àcars. Es metabolitza i s'excreta ràpidament del cos degut a la seva elevada solubilitat en aigua. Per altra banda, el β-HCH és l'isòmer més estable per la seva estructura molecular. Tendeix a acumular-se en els organismes i pot actuar com agent estrogènic (Walker et al., 1999).

Tots els isòmers d'HCH són tòxics per als mamífers. L'Agència Internacional d'Investigació del Càncer (IARC) va classificar el γ-HCH juntament amb la barreja tècnica d'HCHs en el grup 2B com a probable carcinogen humà (IARC, 1987). Degut a la toxicitat i persistència en sòls del lindà, se'n va prohibir o

restringir l'ús. Una exposició crònica a aquest compost s'ha vist associada amb alteracions en els sistemes reproductiu, immunològic, endocrí i neurològic (Willett et al., 1998; Alvarez-Pedrerol et al., 2008; Saeedi Saravi i Dehpour, 2016; Fang et al., 2019).

L'any 2009, alguns dels isòmers de l'HCH (alfa, beta i gamma) es van incloure a la llista de contaminants orgànics persistents del Conveni d'Estocolm (OMS i UNEP, 2009b).

El diclorodifeniltricloroetà (DDT) i els seus metabòlits

El DDT (diclorodifeniltricloroetà) és un nsecticida potent. Es va sintetitzar per primera vegada el 1874 però les seves propietats com a insecticida no es van descobrir fins a la dècada dels 30. Es va utilitzar durant la Segona Guerra Mundial per protegir la gent contra la malària, el tifus i el dengue, entre d'altres malalties contagiades per insectes. Després de la guerra el DDT es va continuar utilitzant per controlar els vectors de les malalties abans esmentades i es va estendre el seu ús a l'agricultura.

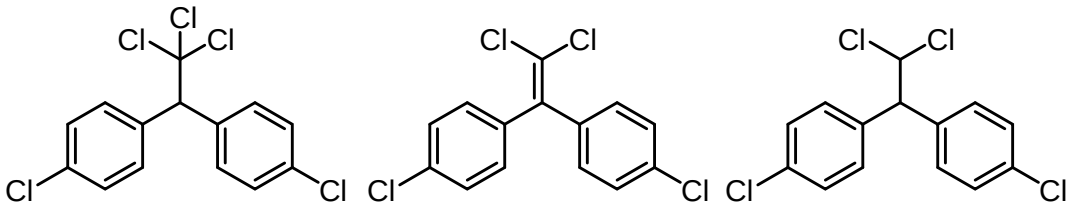

Figura 3.5 Estructures químiques del 4,4'-DDT, 4,4'-DDE i 4,4'-DDD.

La barreja tècnica de DDT conté tres isòmers en la següent proporció: 85% 4,4'-DDT (isòmer més actiu), 15% 2,4'-DDT i traces de 2,2'-DDT.

El DDT es degrada lentament a DDD (diclorodifenildicloroetà) i DDE (diclorodifenildicloroetilè), que són molt persistents i tenen propietats fisicoquímiques similars al compost original (Figura 3.5). Els productes de

degradació també es troben a les barreges tècniques però en concentracions molt baixes.

El DDT es metabolitza a DDE en condicions aeròbiques de biodegradació (procés anomenat deshidroclorinació). La reacció té lloc principalment en organismes vius i està catalitzada per l'enzim deshidroclorinasa. També es pot metabolitzar a DDE o DDD, sota condicions anaeròbiques, en sòls i sediments. Una relació alta de DDE/DDT és, normalment, indicativa d'una contaminació antiga. Per contra, si el valor d'aquesta relació és baix suggereix que pot haver una font recent d'exposició.

Un dels efectes tòxics més coneguts del DDT és l'aprimament de les closques dels ous, que dóna lloc a la pèrdua d'embrions, tot reduint les poblacions d'aus.

Durant la dècada dels 70 es va començar a prohibir el DDT, i el 2001 es va incloure al Conveni d'Estocolm sota l'annex B, per restringir la seva producció i ús al control de vectors (OMS i UNEP, 2001). Tot i així se segueix aplicant en diversos països per lluitar en contra dels vectors de la malària i per prevenir la seva expansió.

L'exposició a llarg termini a DDT s'ha associat amb efectes de salut crònics, tot incloent-hi desordres reproductius, cardiovasculars, metabòlics i neurotoxicitat (Rignell-Hydbom et al., 2009; Al-Saleh et al., 2012; Arrebola et al., 2015; Saeedi Saravi i Dehpour, 2016).

El mirex

El mirex és un pesticida sintètic utilitzat majoritàriament al sud-est dels EEUU (Figura 3.6). S'utilitzava principalment per combatre formigues de foc, a més de per controlar altres tipus de formigues i tèrmts. També es va utilitzar sota el nom de Declorane com a retardant de flama (Faroon et al., 1995; OMS i UNEP, 2001).

El mirex és un compost molt resistent a la degradació, per tant extremadament estable al medi. Es considera un dels pesticides persistents més estables amb una vida mitjana de més de 10 anys (OMS i UNEP, 2001) i la seva fotodegradació resulta en fotomirex, que és tan estable com el producte original (Gandhi et al., 2015).

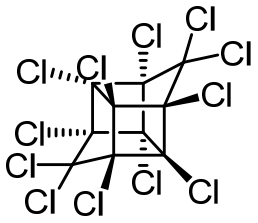

Figura 3.6 Estructura química del mirex.

Es va començar a comercialitzar com a pesticida l'any 1958, es va deixar de produir el 1976 i més tard, el 2001, es va incloure en el Conveni d'Estocolm sota l'annex A, per eliminar la seva producció i ús (OMS i UNEP, 2001).

Encara que no hi havien estudis que provessin que els seus efectes podien ser perjudicials per la salut humana, estudis amb animals van fer que es classifiqués com a possible carcinogen humà. Alguns estudis van provar que era tòxic per algunes espècies de plantes així com per a peixos i crustacis. La ruta d'exposició principal a aquest compost en humans és a través de la dieta, sobretot carn, peix i caça salvatge (OMS i UNEP, 2001).

Els policlorobifenils

Els policlorobifenils són un grup de 209 compostos, anomenats congèneres que estan formats per la cloració de les 10 possibles posicions de l'estructura del bifenil (Figura 3.7).

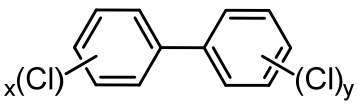

Figura 3.7 Estructura química general dels PCBs.

El grau de cloració així com la posició dels àtoms de clor en la molècula de bifenil determina les seves propietats fisicoquímiques. Per exemple, si les posicions orto (2, 2' i 6, 6') no tenen àtoms de clor l'estructura es manté en forma coplanar, tot definint els PCBs coplanars. Si el compost té un clor en una de les posicions en orto dels anells, aquest s'anomena mono-orto substituït. La resta d'estructures es consideren PCBs no coplanars. Tant els PCBs coplanars com els mono-orto substituïts tenen una configuració que els permet rotar lliurement i adoptar una configuració plana similar a l'estructura de les dioxines. Aquest fet és important a nivell mediambiental i analític perquè aquests són els PCBs més tòxics.

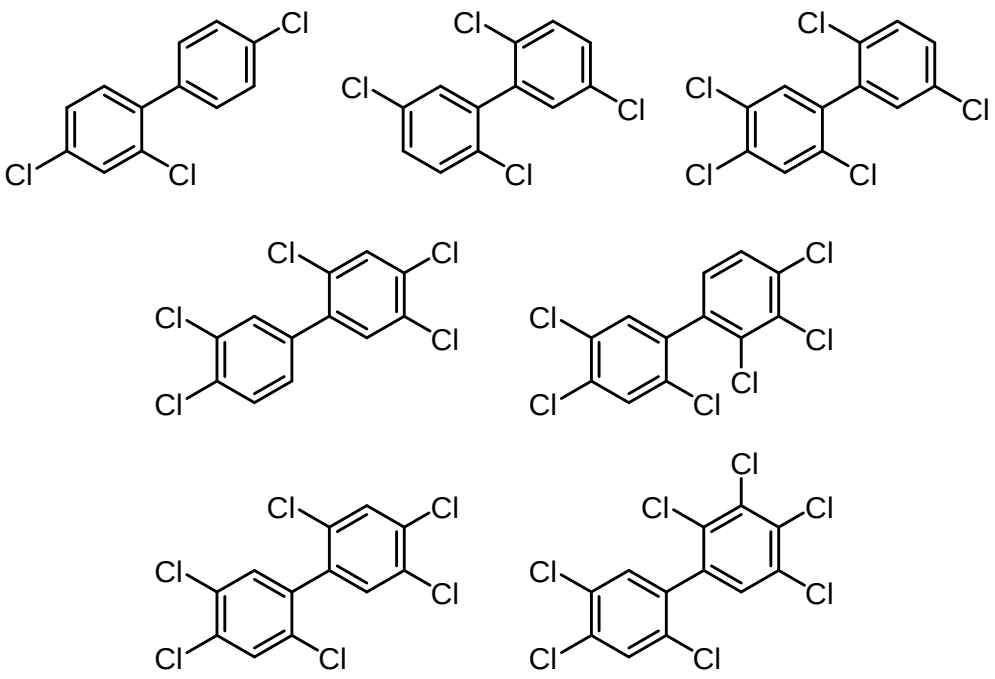

Figura 3.8 De esquerra a dreta i de dalt a baix, estructura química dels congèneres 28, 52, 101, 118, 138, 153 i 180 de PCBs.

Els congèneres de PCB estudiats en aquesta tesi han estat el 28, 52, 101, 118, 138, 153 i 180 (Figura 3.8), perquè són els més abundants en matrius mediambientals i humanes.

Els PCBs es van sintetitzar per primera vegada a Alemanya l'any 1881, mentre que la seva producció industrial no va arribar fins els anys 30. L'ús d'aquests compostos es va expandir ràpidament gràcies a les seves propietats, que comportaven una estabilitat química alta, una inflamabilitat baixa, una conductivitat elèctrica i propietats aïllants baixa, una resistència a l'oxidació per àcids alta i una solubilitat en aigua baixa, entre d'altres. Totes aquestes propietats els han fet molt útils per ser utilitzats en intercanviadors de calor, transformadors i condensadors en sistemes elèctrics, plastificants en pintures, pigments i plàstics entre moltes d'altres aplicacions comercials i industrials (Hutzinger et al., 1974).

La seva incorporació al medi ambient s'atribueix a la difusió per l'atmosfera o la filtració a aigües subterrànies després de la seva aplicació. El punt màxim de producció d'aquests compostos va ser durant el final de la dècada dels 70, però aviat es va trobar que eren uns compostos perillosos pel medi ambient. Així doncs, els PCBs es van incloure al Conveni d'Estocolm el 2001 (OMS i UNEP, 2001).

L'any 1968 a Kyushu (Japó) va haver un cas de contaminació per PCBs, degut al consum d'oli contaminat. Va donar lloc a una malaltia estranya, anomenada *Yusho*, caracteritzada per l'aparició d'erupcions tipus acne (cloracne), hiperpigmentació de la pell i secreció dels ulls. A més, els nens exposats a PCBs durant el període de gestació o durant la lactància van presentar un retard important en el creixement intrauterí, un lleuger retard mental posterior així com una hiperpigmentació de la pell (Fujiwara, 1975).

Els PCBs són tòxics pels peixos, a dosis baixes els afecta en la seva capacitat de reproducció mentre que a dosis altes els poden arribar a matar. Nens de mares que menjaven molt de peix del llac Michigan, als Estats Units, van resultar tenir pitjor memòria a curt termini. Els PCBs estan classificats com probables carcinògens (Grup 2A) per la IARC (IARC, 1987). A més, poden actuar com

disruptors endocrins, tot causant alteracions en el sistema nerviós i reproductiu, desordres hepàtics, immunotoxicitat i alteracions de les funcions tiroides, perquè tenen una estructura similar a la tiroxina (hormona tiroide T4).

Els compostos organobromats

Els polibromodifenil èters (PBDEs)

Els polibromodifenil èters (PBDEs) són un grup constituït per 209 congèneres que varien segons el número i la posició dels àtoms de brom a l'estructura del difenil èter (Figura 3.9). Com que les estructures són anàlogues a les dels PCBs però amb àtoms de brom en lloc de clor, s'utilitza la mateixa nomenclatura (Ballschmiter i Zell, 1980). Aquest grup de compostos s'han utilitzat com a retardants de flama de manera molt extensiva en productes industrials i aplicacions comercials durant vàries dècades.

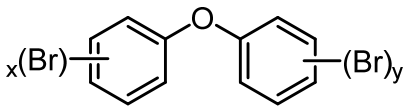

Figura 3.9 Estructura química general dels PBDEs.

Els PBDEs s'han distribuït en tres barreges comercials de congèneres amb nivells de bromació diferents (penta-BDE, octa-BDE i deca-BDE).

La barreja de penta-BDE està composta principalment dels congèneres 47, 99 i 100 i s'utilitzava com additiu a les espumes de poliuretà en mobles, catifes o matalassos. En la barreja d'octa-BDE predominava el BDE-183 seguit del BDE-153 i el BDE-154 i s'utilitzava principalment en termoplàstics, com poliestirè d'alt impacte.

Finalment, el deca-BDE era bàsicament decabromodifenil èter (BDE-209), s'utilitzava predominantment en roba, així com en components plàstics per a productes electrònics (televisors, ordinadors). D'acord amb la demanda del

mercat global de PBDE, el 2001, predominava la fabricació de BDE-209 (83%), seguit de penta-BDE (11%) i octa-BDE (6%) (La Guardia et al., 2006).

Els congèneres estudiats en aquesta tesi doctoral han estat els següents: 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190 i 209 (Figura 3.10) que són els més comuns en matrius mediambientals i humanes.

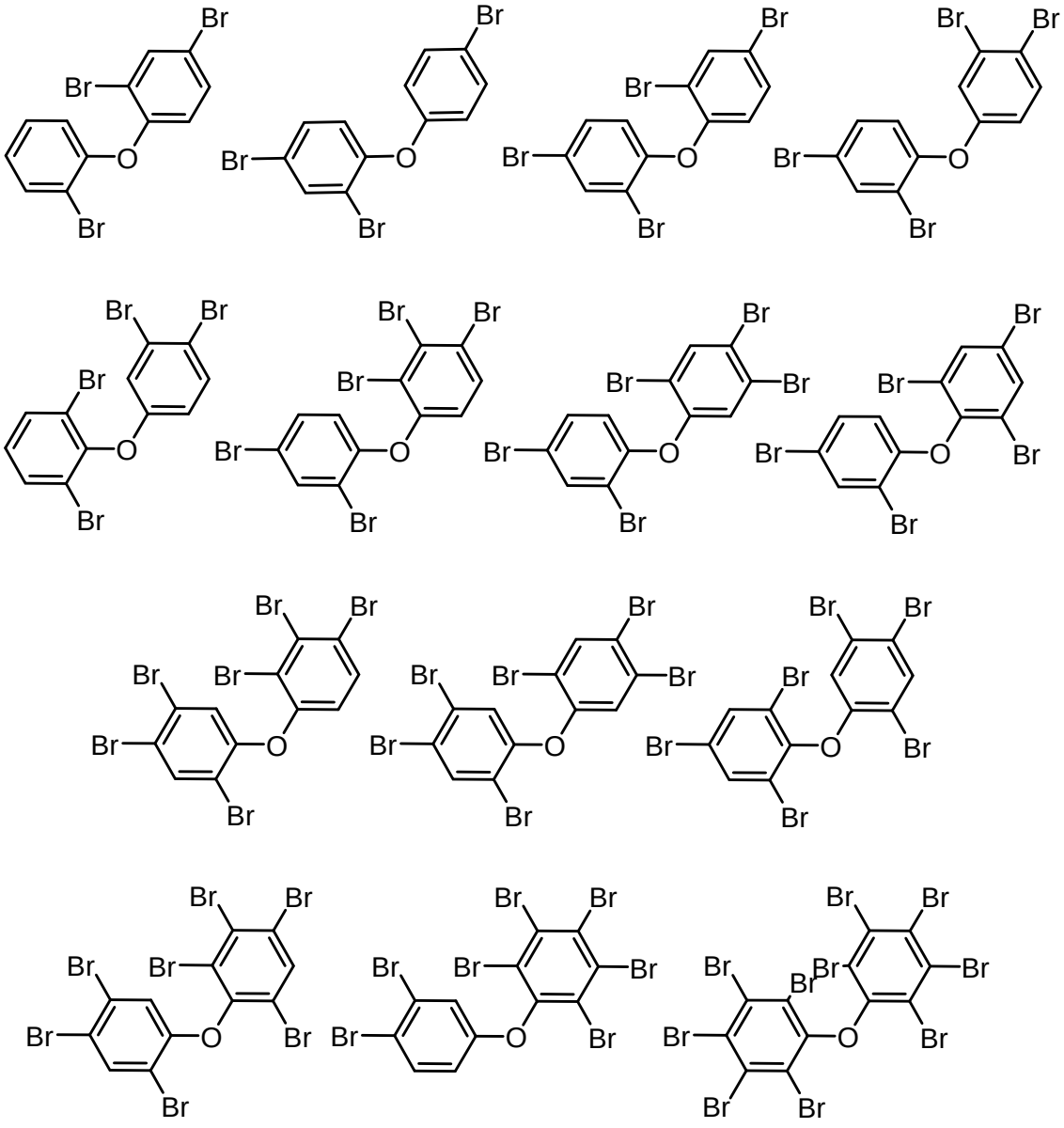

Figura 3.10 De esquerra a dreta i de dalt a baix, estructura química dels congèneres 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190 i 209 de PBDEs.

La preocupació al camp de la salut pública ha anat creixent amb els anys degut als potencials efectes en la salut que pot provocar una exposició crònica a aquests compostos. Conseqüentment, la Unió Europea va prohibir les formulacions penta i octabrominades el 2004. Més tard, el 2009, aquests van ser inclosos al Conveni d'Estocolm (OMS i UNEP, 2009b).

Per altra banda, encara que la producció i ús del deca-BDE està permesa, cada vegada està més restringida a usos molt puntuals com per exemple transports aeris. No obstant, la degradació del BDE-209 dóna com a subproductes congèneres amb grau més baix de bromació que són més tòxics en el medi ambient (Ross et al., 2009).

La preocupació per l'exposició a aquests compostos ve donada per la seva toxicitat potencial com a disruptors endocrins i els efectes sobre el neurodesenvolupament en nadons, entre altres (Akutsu et al., 2008; Herbstman et al., 2008; Herbstman et al., 2010).

Exposició humana a COPs

Els compostos presentats anteriorment estan o van estar presents en molts productes utilitzats diàriament, com a conseqüència es troben tant en teixits com en fluids humans (per exemple, teixit adipós, fetge o sang).

L'exposició a COPs por ocórrer de diferents formes. La dieta és la ruta més important (representant <90% de l'exposició total) i, especialment a través del consum de menjar contaminat amb contingut en greixos alt com peix gras, carn vermella o aus, entre d'altres (Domingo, 2012; Xu et al., 2017; Junque et al., 2018). No obstant, un cop ingerits part d'aquests compostos es poden excretar per la femta (To-Figueras et al., 2000), perquè no sempre s'absorbeixen totalment absorbits a l'intestí (Kreuzer et al., 1997; Moser i McLachlan, 2001).

Una altra ruta menys important per a la incorporació de COPs al cos humà és per inhalació, tant en llocs tancats com a l'exterior, i per contacte dèrmic.

Aquest és el cas de la població de Flix, que va estar exposada a grans quantitats d'OCs emesos per una planta clor-alcalí, concretament HCB, un subproducte de la síntesi de dissolvents organoclorats. Alguns estudis, realitzats a la població de Flix, van mostrar els nivells més alts d'aquest compost que s'havien trobat en mostres humanes, tot indicant la seva incorporació per via respiratòria (Sala et al., 1999). De la mateixa manera, la inhalació de pols és un de les vies més importants de l'exposició a PBDEs (de la Torre et al., 2018), en els ambients interiors a casa, l'escola o el lloc de treball.

Els COPs s'emmagatzemen als teixits grassos degut a la seva lipofilicitat i la seva resistència a la degradació. L'acumulació que s'observa per part d'aquests compostos en teixits humans suggereix les persones tenen un metabolisme ineficient per a la seva excreció, el que afavoreix la bioacumulació continuada amb l'edat.

El metabolisme hepàtic juga un paper molt important en l'eliminació de moltes substàncies químiques (Dayton et al., 1983). La transformació dels COPs condueix a la seva incorporació als fluids biliars o el plasma, tot permetent la seva eliminació parcial a través del sistema excretor (Moser i McLachlan, 2001; Genuis et al., 2016).

Hi ha evidències d'una major eliminació de COPs en dones en comparació amb els homes, associades a la reproducció. Per exemple, durant la gestació aquests compostos es transfereixen de la mare al fetus per la placenta i un cop el nen és nat es continuen transferint mitjançant la llet materna (Vizcaino et al., 2014; Gascon et al., 2015; Lopez-Espinosa et al., 2015). Tant l'embaràs com el part o la lactància constitueixen una via d'eliminació de COPs per a la dona.

A més de les rutes d'exposició a les que estem exposats en la vida diària, les persones també podem estar exposades en el lloc de treball o per accident. Una exposició crònica, fins i tot en condicions d'exposició lleu, pot generar un marge ample de problemes de salut, des d'una malaltia fins a la mort (Gascon et al.,

2013; Govarts et al., 2018). No obstant, els efectes produïts per una exposició prolongada a nivells baixos de COPs no es coneixen de forma completa i mereixen més atenció en estudis amb població general i vulnerable, com són els nens o les dones embarassades.

Poblacions d'estudi

En aquest treball s'han estudiat tres poblacions de dones diferents. Per una banda, dos poblacions de dones que acabaven de donar a llum de Salta i Ushuaia, dues ciutats de l'Argentina i per altra banda, una població de dones embarassades del Districte Autònom de Chukotka, Russia. Ambdós exemples corresponen a casos molt poc estudiats. Les dues ciutats de l'Argentina es troben a l'hemisferi sud, pràcticament no hi ha estudis de l'acumulació de COPs en poblacions generals d'aquest hemisferi. La regió de Chukotka es troba a l'extrem est de Sibèria, novament, la informació sobre la presència de COPs en aquesta zona del planeta és molt minsa.

Salta i Ushuaia - Argentina

Argentina és un estat de l'Amèrica del Sud integrat per vint-i-tres províncies i una ciutat autònoma, Buenos Aires, que és la capital. Limita al nord amb Bolívia i el Paraguai, a l'est amb el Brasil, l'Uruguai i l'oceà Atlàntic i a l'oest amb Xile (Figura 3.11). Amb una superfície de 2.766.890 km^2 és el vuitè país més gran del món. L'any 2011, quan es van recollir les mostres, hi vivien uns 41 milions de persones.

A causa de la seva extensió, des de la meitat de l'Amèrica del Sud fins a l'Antàrtida, trobem que l'Argentina contempla un dels paisatges i climes més diversos del món.

Es pot dividir en tres àrees geogràfiques clarament diferenciades. Per una banda, les planes fèrtils de les Pampes, al centre del país i les quals són el centre

de la riquesa agrícola de l'Argentina, des del centre cap al sud a la Terra del Foc tenim l'altiplà de la Patagònia i finalment, la cadena muntanyosa dels Andes a l'oest, formant frontera amb Xile. Entre el punt més el sud de l'Argentina i el de més el nord hi ha una diferència latitudinal de 34°. Com a conseqüència la seva diversitat climàtica és excepcional, variant des de clima subtropical al nord fins a subpolar al sud.

Figura 3.11 Localització geogràfica d'Argentina a Amèrica del Sud (esquerra) i localització de les províncies de Salta i Terra del Foc a l'Argentina, marcat en taronja les ciutats de Salta i Ushuaia (dreta).

Les dues ciutats estudiades tenen la característica distintiva de ser dos llocs del mateix país localitzats en aquestes dues latituds extremes (54°S Ushuaia i 24°S Salta). La temperatura mitjana diària d'Ushuaia varia des de 1,5 fins a 9,4 °C, que d'acord amb la classificació climàtica de Köppen correspon a un

clima de tundra, característic de zones polars Àrtiques i Antàrtiques (Luchini i Wicki, 2002). En contraposició, la diversitat del relleu de Salta determina l'existència de diversos microclimes. A la regió on hi viu la majoria de la població saltenya la temperatura mitjana és de 20 °C, que correspon a un clima temperat, amb pluges estacionals que penetren profundament als sòls, tot dotant-los una gran fertilitat (Romero i González, 2012).

Salta està localitzada en la part més septentrional d'Argentina i la seva població es troba distribuïda de manera desigual. La zona amb més densitat de població és la Vall de Lerma, on es troba la ciutat de Salta, capital de la província. Segons el cens, el 2010 hi vivien 1,2 milions de persones, de les quals un 87% vivien en zones urbanes (INDEC, 2010). Encara que la majoria de dones voluntàries d'aquest estudi provenen de la ciutat de Salta, algunes són d'altres ciutats de la província.

La província de Salta està centrada en economies primàries i compta amb una agricultura molt diversificada com a conseqüència de la seva heterogeneïtat d'ambients productius. També posseeix una enorme riquesa minera, part del seu desenvolupament està lligat a jaciments de petroli i gas, a més d'altres tipus de mines com ara sofre, marbre, coure, zinc, plata, manganès, ferro, quars o mica. Alhora l'activitat industrial també està molt diversificada i es localitza en tot el territori provincial. Entre les industries més destacades trobem la refineria de petroli, sucre, processament de fusta, manufactura de tabac o la industria del metall. A més, també és important la indústria artesanal de cuir, ferro, plata, teixits i terrisseria.

Per contra, la badia d'Ushuaia està localitzada al canal de Beagle, a la província Terra del Foc. La ciutat d'Ushuaia és l'únic assentament urbà en la costa més meridional de l'illa Terra del Foc (Commendatore et al., 2012). Segons el cens, el 2010 la població total d'Ushuaia era de 56.593 (INDEC, 2010). En

aquest cas totes les dones que han participat en aquest estudi són de la ciutat d'Ushuaia.

L'economia local depèn bàsicament del turisme, el mercat i el desenvolupament industrial, que suposa un tràfic marítim intens al voltant del port comercial. El combustible hi arriba per transport marítim i s'acumula en tancs a prop de la costa (Amin i Comoglio, 2002). La badia d'Ushuaia s'ha identificat com una regió adequada pel desenvolupament d'aqüicultura (Luchini i Wicki, 2002) i degut a la seva biodiversitat es va designar com a Zona de Protecció Especial (PNA, 1998).

Un aspecte important de l'interès de l'estudi d'aquestes poblacions és que es troben a l'hemisferi sud. Els COPs s'han sintetitzat i utilitzat majoritàriament a l'hemisferi nord, però la seva capacitat per ser transportats a través de l'atmosfera els ha distribuït per tot el planeta (Simonich i Hites, 1995), fins i tot a l'hemisferi sud (Amin et al., 2011). La informació disponible en poblacions humanes de l'hemisferi sud és molt limitada i amb aquest estudi es vol contribuir a ampliar-la. Finalment, el disseny de l'estudi permet comparar dues ciutats d'un mateix país però amb condicions climàtiques molt diferents. Això permet observar si les diferències latitudinals són imoptants en relació a la contaminació d'amdues ciutats.

Districte Autònom de Chukotka - Rússia

Rússia és un estat transcontinental d'Euràsia i està integrat per diferents subjectes federals. Limita a l'oest amb Noruega, Finlàndia, Estònia, Letònia, Lituània, Polònia, Bielorússia i Ucraïna, al sud amb Geòrgia, Azerbaidjan, Kazakhstan, la República Popular de la Xina, Mongòlia i Corea del Nord. A més, l'extrem oriental del país és molt a prop d'Alaska (EEUU). Té una superfície de 17 milions km^2, fet que el fa el país més extens del món.

Chukotka és un dels quatre districtes autònoms que es poden trobar a Rússia i la seva capital és Anádyr. Està ubicat a l'extrem nord-est del país i separat d'Alaska per l'estret de Bering. Aproximadament el 50% del territori està per sobre del Cercle Polar Àrtic (Figura 3.12). Té una àrea de 737.000 km^2 i una població de 51.286 segons el cens de 2010, essent el 35% població indígena (GKS, 2011). El Districte Autònom de Chukotka està dividit en sis districtes i en aquest estudi han participat voluntàries de tots sis. Les localitzacions en detall es mostren a la Fig. 1 de l'ARTICLE 3.

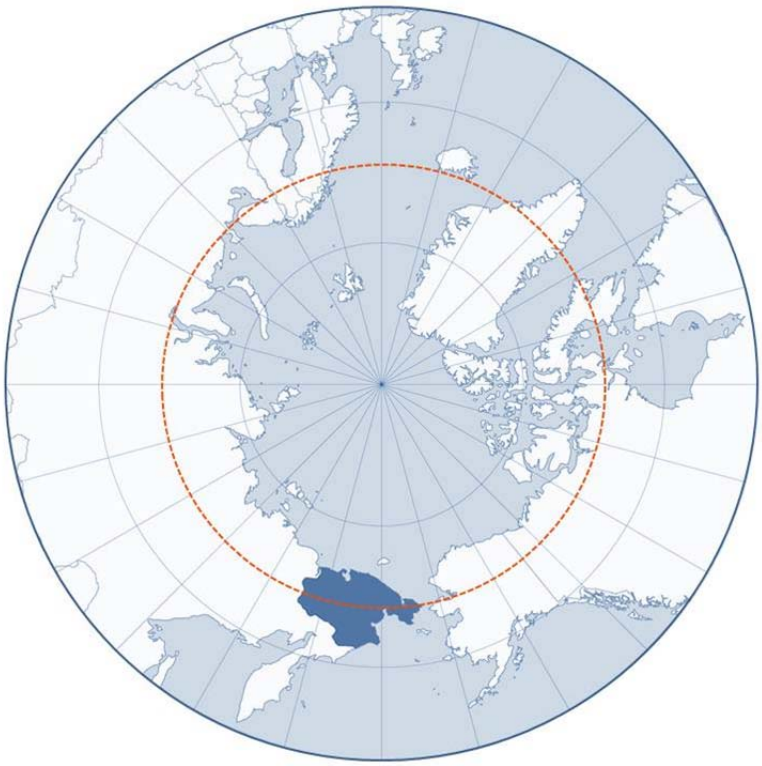

Figura 3.12 En blau fosc la localització de Chukotka a l'Àrtic. Delimitat en taronja el Cercle Polar Àrtic.

La regió té grans reserves de petroli, gas natural, carbó, or i tungstè, que s'exploten gradualment, encara que aquesta no és l'activitat econòmica més popular. La major part de la població viu de la ramaderia de rens, de la caça i la pesca. La pesca és una de les activitats tradicionals principals entre els indígenes de la zona, el seu objectiu principal són les balenes, morses i foques

que els proveeixen de carn per la seva alimentació, greix i pell. En canvi, la població urbana es dedica generalment a la mineria, l'administració, la construcció, l'art i cultura, l'educació o la medicina (AMAP, 2004).

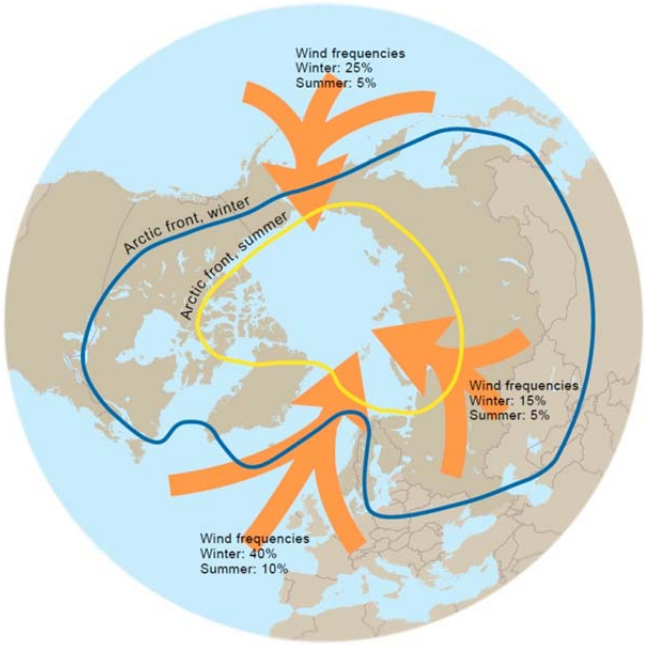

Figura 3.13 Posició mitjana de les masses d'aire a l'Àrtic al gener i juliol, i les freqüències de vents a l'hivern i l'estiu (AMAP, 1997).

El clima de la Rússia àrtica es caracteritza per l'absència de radiació solar durant l'hivern que dona lloc a temperatures molt baixes i una radiació solar significativa a l'estiu, tot i que degut a que part de l'energia s'empra per fondre el gel, les temperatures no arriben a ser molt altes. Com a conseqüència, els corrents atmosfèrics de l'Àrtic difereixen entre l'estiu i l'hivern. Durant l'hivern les masses d'aire es mouen cap a l'Àrtic des d'Europa en direcció nord-est, o des de l'Àsia central i Sibèria. A l'estiu els sistemes d'alta pressió continental desapareixen i els sistemes de baixa pressió oceànica es debiliten, canviant d'aquesta manera els influxos d'aire respecte l'hivern. En conjunt (Figura 3.13), durant l'estiu la component nord de transport atmosfèric és més freqüent en totes les regions de la Rússia àrtica excepte Chukotka. En canvi, durant l'estiu,

Chukotka està afectada bàsicament pel transport tant de l'Oceà Pacífic, l'Àsia Oriental i la part més a l'oest de Rússia.

Degut a la naturalesa de la circulació atmosfèrica, les emissions locals d'Europa i Àsia de compostos organoclorats i organobromats, entre d'altres, juguen un paper molt important en la contaminació de l'Àrtic. En el passat, durant el projecte abans esmentat es van trobar nivells d'OCs extremadament alts en sèrum de dones de Chukotka (Sandanger et al., 2003). A més, l'alimentació tradicional a base de grans mamífers entre la població de Chukotka fa que aquesta població estigui més exposada a contaminants en comparació amb altres de la Rússia àrtica que segueixen una alimentació menys tradicional. És important estudiar la contaminació de la població nativa de Chukotka per entendre millor quins són els factors que determinen l'exposició i quins efectes poden tenir, en el cas de dones embarassades, sobre els seus nadons.

3.2 Metodologia

Disseny dels estudis

El treball realitzat amb dones de l'Argentina està emmarcat en l'estudi EMASAR (Estudio del medio Ambiente y la Salud Reproductiva). És un estudi observacional, amb un disseny transversal emmarcat en el context d'AMAP sota el *Circumpolar Programme* (AMAP, 2018). Es va dissenyar per investigar els riscos de salut per a dones i fetus relacionats amb la seguretat alimentària i l'exposició a substàncies tòxiques persistents en dos regions de l'Argentina (Økland et al., 2017).

Respecte a les mostres de Russia, s'ha avaluat l'estat actual d'unes de les regions estudiades fa 15 anys al projecte "*Persistent toxic substances (PTS), food security and indigenous peoples of the Russian North"*, que estava dissenyat per

avaluar l'abast de la contaminació per substancies tòxiques persistents a la Rússia àrtica, determinar els efectes que tenen sobre la població indígena i identificar mesures per millorar la situació de la regió (AMAP, 2004).

Recol·lecció de dades

Qüestionaris i informació clínica

Les participants es sotmetien a un examen mèdic i una entrevista per part d'un obstetra qualificat. Aquests qüestionaris proporcionen informació sobre algunes variables sociodemogràfiques com ara edat, lloc de naixement, paritat, nivell educatiu i econòmic o hàbits de consum d'alcohol de mares i pares.

L'examen mèdic proporciona informació sobre l'edat gestacional, dades antropomètriques tant de mares com de nens i si hi ha alguna malformació notable en el nounat.

Presa de mostra

Les mostres de sang venosa es van extreure de la vena cubital de les mares, sense necessitat de estar en dejú. Per l'anàlisi dels compostos organoclorats i organobromats la sang es recull en un BD Vacutainer® per després ser transferida en un crio-vial i centrifugada a 2000 rpm durant 10 min. Amb aquesta centrifugació s'aconsegueix separar el sèrum dels glòbuls vermells. El sèrum és equivalent al plasma però sense les proteïnes anticoagulants que podrien interferir en les anàlisis. En aquest punt, el sèrum es transfereix a un vial de vidre i es conserva congelat a -20 °C fins el moment d'analitzar-lo (Økland et al., 2017).

Com a anàlisis de rutina als hospitals es determina el perfil lipídic de cada una de les mostres: colesterol, triglicèrids, fosfolípids i lipoproteïnes d'alta i baixa densitat (HDL i LDL). Algunes d'aquestes mesures són necessàries pel

càlcul del total de lípids, i normalitzar els resultats obtinguts de les anàlisis respecte a aquest paràmetre.

Determinació d'OCs i PBDEs

En les seccions següents es detalla el protocol analític que es va seguir per analitzar els compostos amb la màxima eficiència i minimitzar la contaminació externa de les mostres, així com la metodologia emprada en la preparació dels estàndards i les tècniques de separació i quantificació utilitzades.

Materials, dissolvents, reactius i estàndards

Neteja del material

El material de vidre de laboratori es neteja amb sabó, després es sonica durant 15 min en un bany d'ultrasons amb detergent alcalí (Extran, AP-13). A continuació, s'esbandeix amb aigua Milli-Q i acetona i després es deixa assecar al forn a 80 °C. Un cop sec, tot el material (inclús aquell que no es pot rentar, com les pipetes Pasteur), es deixa a la mufla durant tota la nit a 400 °C.

El sulfat de sodi i la llana de vidre es netegen en Soxhlet durant 6 hores amb una solució de diclorometà:n-hexà (1:1 v/v). Posteriorment s'assequen sota una llum infraroja. El sulfat de sodi es deixa tota la nit a la mufla a 400 °C.

Dissolvents i reactius

Els dissolvents emprats en l'extracció i preparació de les mostres han estat isooctà, n-hexà, diclorometà i acetona, tots ells de Merck (Darmstakt, Alemanya) per l'anàlisi a nivell traça de compostos orgànics. L'àcid sulfúric concentrat i el sulfat de sodi també eren de Merck (Darmstackt, Alemanya). La llana de vidre era de Panreac (Barcelona).

Estàndards

Els estàndards de recuperació tetrabromobenzè (TBB) i PCB-209 permeten mesurar les pèrdues d'anàlit que hi pot haver durant el procés d'extracció. Ambdós compostos són de Dr. Ehrenstorfer GmbH (Augsburg, Alemanya).

Els estàndards interns (PCB-142, BDE-118 i $^{13}C_{12}$-BDE-209) ens permeten controlar la injecció al cromatògraf de gasos. El PCB-142 es va adquirir a Dr. Ehrenstorfer (Augsburg, Alemanya), mentre que el BDE-118 i el $^{13}C_{12}$-BDE-209 són de Cambridge Isotope Laboratories, Inc. (CIL; Andower, MA, USA).

La solució amb la mescla dels estàndards dels OCs i dels PBDEs es prepara a concentracions diferents per obtenir les corbes de calibratge. Per a l'anàlisi dels OCs, s'utilitzen els estàndards de pentaclorobenzè (PeCB), hexaclorobenzè (HCB), hexaclorociclohexans (α-, β-, γ-, δ-HCHs) 2,4'-DDT, 4,4'-DDT i els seus metabòlits (2,4'-DDD, 4,4'-DDD, 2,4'-DDE i 4,4'-DDE), mirex i policlorobifenils (congèneres de PCB 28, 52, 101, 118, 138, 153 i 180), tots ells de Dr. Ehrenstorfer (Augsburg, Alemanya). La mescla de congèneres predominants de polibromodifenil èters per a l'anàlisi de PBDEs s'adquireix de Cambridge Isotope Laboratories, Inc. (CIL; Andover, MA, USA). La solució inclou els congèneres següents: 17, 28, 46, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190 i 209.

Procediment experimental: Extracció líquid-líquid

Les mostres de sèrum es descongelen i homogeneïtzen amb un vòrtex. De cada mostra s'agafa una alíquota d'1 mL que es fica en un tub de centrifuga de 10 mL, a cadascun s'afegeixen 25 µL de la solució d'estàndards de recuperació que conté TBB i PCB-209 (50 ng/mL). Després, s'afegeixen 3 mL d'*n*-hexà i 2 mL d'H_2SO_4, es barregen amb l'ajuda d'un vòrtex (1,500 rpm, 30 s) i centrifuguen (3,500 rpm, 5 min), tot formant-se dues capes, una inferior on queda el sèrum i l'àcid sulfúric i una superior on tenim l'hexà amb els anàlits a mesurar.

L'hexà sobrenedant es transfereix amb l'ajuda d'una pipeta Pasteur a un segon tub de centrífuga. Al tub amb H_2SO_4 s'afegeixen 2 mL més d'hexà, es barreja i es centrifuga, aquest últim pas es repeteix un cop més fins a obtenir un total de 7 mL d'hexà. A aquest s'afegeixen 2 mL d'H_2SO_4. A continuació la suspensió es barreja en vòrtex (1,500 rpm, 60 s), es centrifuga (3,500 rpm, 10 min), i l'hexà sobrenedant es passa a través d'una columna amb llana de vidre i sulfat de sodi a un tub cònic. El sulfat de sodi reté traces d'àcid que no s'ha pogut separar en l'extracció i que podria fer malbé la columna capil·lar cromatogràfica.

Els extractes d'hexà recol·lectats en el tub cònic s'evaporen gairebé fins a sequedat sota un raig de nitrogen ultra pur, amb una pipeta Pasteur es transfereixen a un vial apte per a cromatografia, s'evaporen a sequedat i s'afegeixen 100 µL de l'estàndard intern PCB-142 en iso-octà (10 ng/mL) (Grimalt et al., 2010).

Després de l'anàlisi dels OCs per GC-ECD (detallat a la secció d'anàlisi instrumental), s'evaporen un cop més sota nitrogen els extractes de les mostres. Llavors, s'afegeixen 20 µL de BDE-118 (20 ng/mL) i 10 µL de $^{13}C_{12}$-BDE-209 (6,5 ng/mL) per a la determinació de PBDEs (Vizcaino et al., 2009).

Anàlisi instrumental

Condicions cromatogràfiques per l'anàlisi de OCs

L'anàlisi dels OCs s'ha dut a terme per cromatografia de gasos amb detector de captura d'electrons (GC-ECD, Agilent Technologies 7890A). La columna utilitzada per a la separació dels compostos d'interès va ser una DB-5 (60 m de llarg i 0,25 mm de diàmetre intern; Agilent J&W Scientific, Folsom, CA, USA), amb rebliment de 5% de fenil-metilpolisiloxà (0,25 µm de gruix).

L'heli i el nitrogen són, respectivament, el gas portador (a un flux constant de 1,5 mL/min) i el gas auxiliar del detector a 60 mL/min. La injecció (2 µL) és automàtica en mode *splitless* (*split* tancat durant 1,5 min) a 280 °C i temperatura

del detector a 310 °C. El programa de temperatura del forn comença a 90 °C i es manté durant 2 min (Figura 3.14). Després, es puja a 15 °C/min fins a 130 °C i fins a 290 °C a 4 °C/min que es mantenen durant 20 min. El temps total d'injecció per mostra és de 64,67 minuts (Carrizo i Grimalt, 2009).

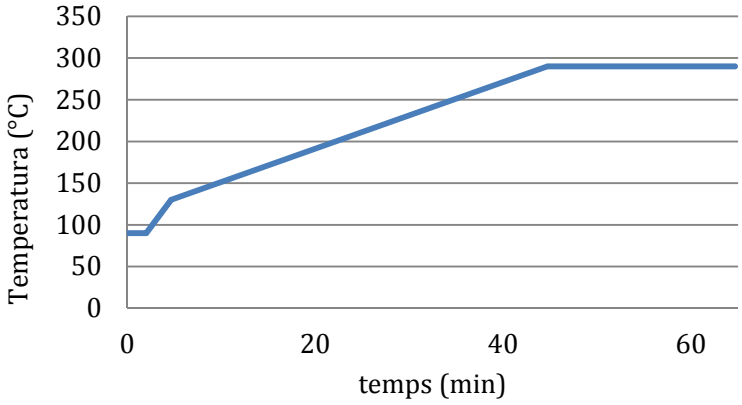

Figura 3.14 Esquema de la rampa de temperatura del GC-ECD per a la separació dels compostos organoclorats.

A la Figura 3.15 es mostra la separació dels compostos organoclorats d'interès, així com els estàndards de recuperació i intern. Els compostos s'identifiquen segons el seu temps de retenció (tR).

Condicions cromatogràfiques per l'anàlisi de PBDEs

Els polibromodifenil èters es determinen per cromatografia de gasos (GC, Agilent Technologies 6890N) acoblat a un espectròmetre de masses (EM, Agilent Technologies, 5975N) funcionant en mode d'ionització química negativa (NICI). L'instrument s'equipa amb una columna capil·lar de sílice fosa de baix sagnat DB-5MS (15 m de llarg, 0,25 mm de diàmetre intern i 0,10 µm de gruix).

S'utilitza un cop més heli com a gas portador i amoníac com a gas reactiu per l'anàlisi a l'espectròmetre de masses. La injecció es duu a terme en mode *splitless.* La temperatura inicial al forn és de 90 °C, que es manté durant 1,5 min, seguidament es fa una primera rampa a 20 °C/min fins a 200 °C, una segona fins

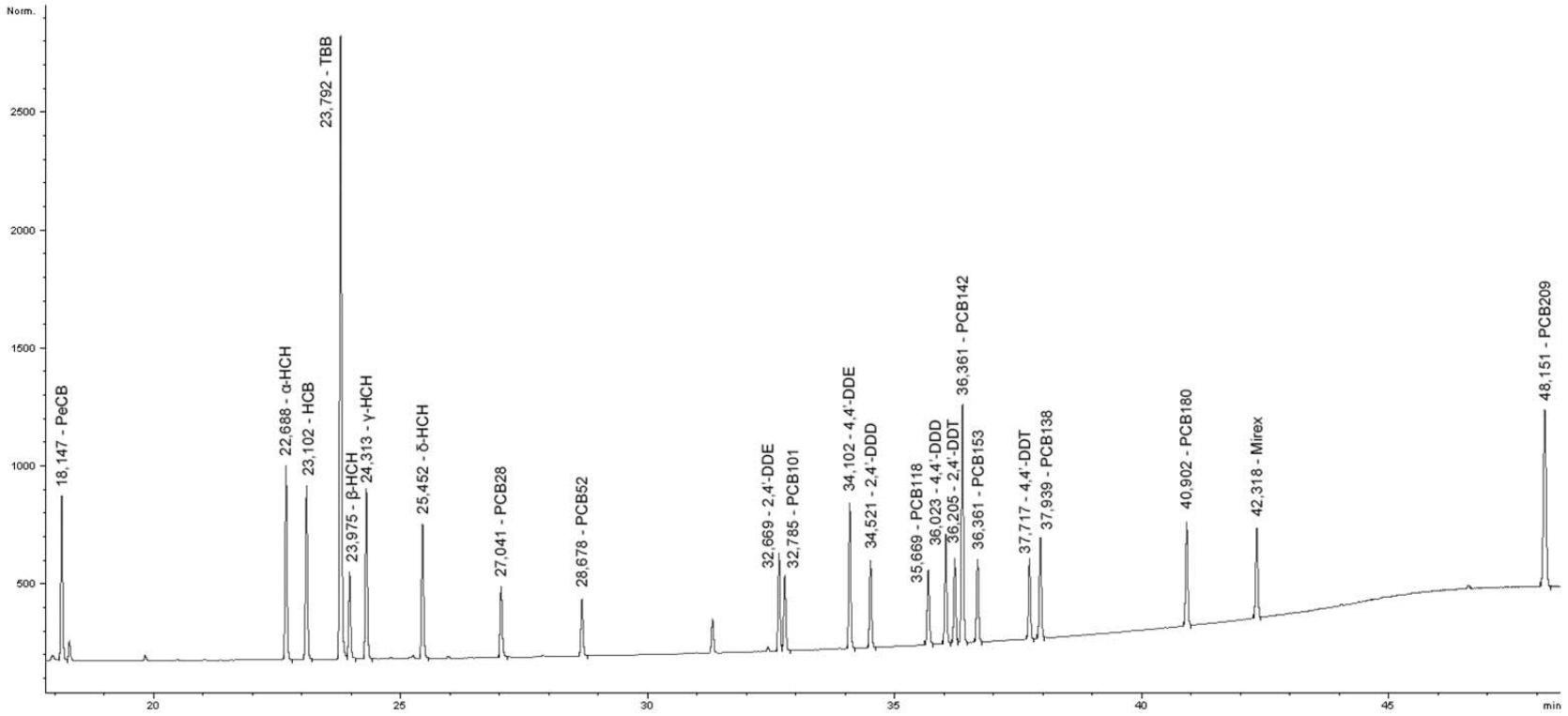

Figura 3.15 Cromatograma mostrant els pics d'interès (PeCB, α-HCH, HCB, β-HCH, γ-HCH, δ-HCH, PCB-28, PCB-52, 2,4'-DDE, PCB-101, 4,4'-DDE, 2,4'-DDD, PCB-118, 4,4'-DDD, 2,4'-DDT, PCB-153, 4,4'-DDT, PCB-138, PCB-180 i mirex) els patrons de recuperació (TBB i PCB-209) i el patró intern (PCB-142).

a 275 °C a 5 °C/min i una tercera fins a la temperatura final de 300 °C a 30 °C/min, aquesta darrera temperatura es manté durant 10 min (Figura 3.16). El temps total d'injecció és de 32,82 minuts (Vizcaino et al., 2009).

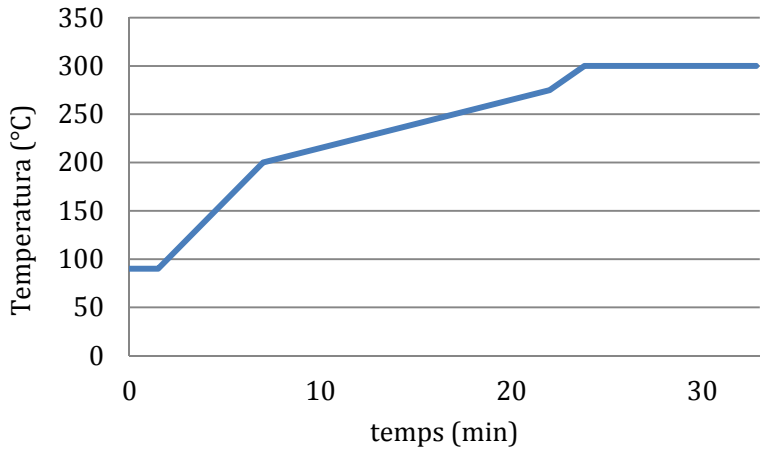

Figura 3.16 Esquema de la rampa de temperatura del GC-NICI MS per a la separació dels compostos organobromats.

La identificació dels PBDEs es basa en el seu temps de retenció (tR) i la informació de massa, a partir del registre en mode de monitorització selectiva d'ions (SIM), que utilitza la relació massa carrega (m/z) dels ions de brom, 79/81, per tots el congèneres, excepte m/z 487/489 pel BDE-209 i m/z 495/497 pel $^{13}C_{12}$-BDE-209. A la Figura 3.17 es mostra un cromatograma amb els compostos d'interès i els estàndards.

Control de qualitat de les anàlisis

Per avaluar l'exactitud i precisió del procediment analític i la metodologia emprada en els estudis s'han revisat alguns paràmetres.

Blancs de procediment

S'han realitzat blancs de procediment per cada sèrie de mostres de sèrum (cada 15 mostres). La quantificació dels blancs s'ha fet servir tant per controlar contaminacions possibles relacionades amb l'ambient del laboratori, material o

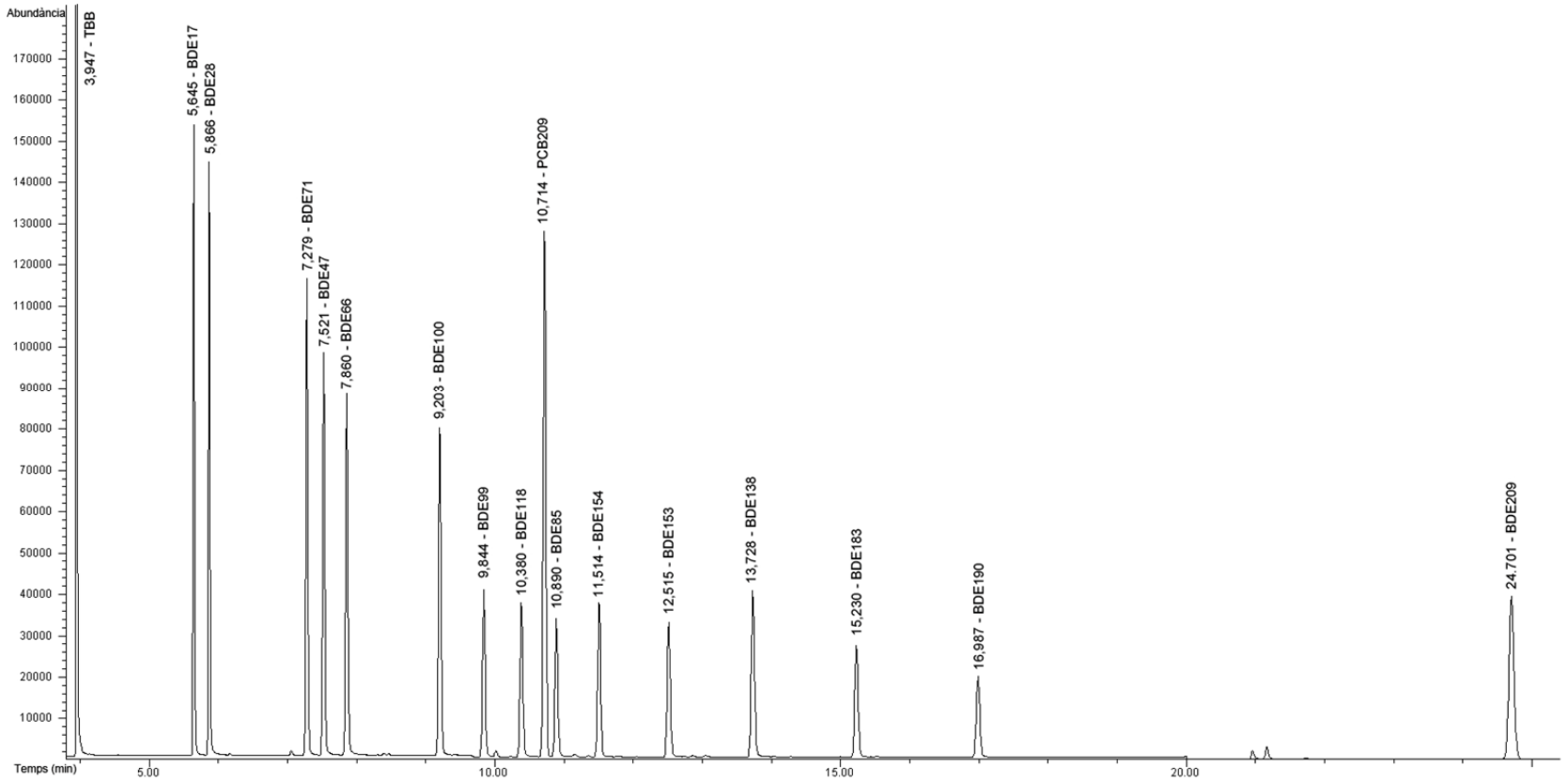

Figura 3.17 Cromatograma mostrant els pics d'interès (congèneres de BDE 17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138, 183, 190, 209), els patrons de recuperació (TBB i PCB-209) i el patró intern (BDE-118).

dissolvent, com per calcular els límits de detecció i quantificació dels OCs i PBDEs. En general, les concentracions per compostos organohalogenats trobats als blancs corresponen a una contaminació mínima del procediment analític i no modifica els resultats finals.

Recuperació dels estàndards

Com s'ha explicat abans, al començament del procés d'extracció s'afegeix una concentració coneguda dels estàndards de recuperació. El càlcul del tant per cent de recuperació no és només per controlar que el mètode és apropiat pels analits sinó també per corregir les petites pèrdues que hi pugui haver al llarg de tot el procediment.

En l'anàlisi dels compostos organoclorats la mitjana de les recuperacions (±σ) del TBB i el PCB-209 van ser 60±18% i 85±18%, respectivament. Les respostes relatives del PCB-142 en cada injecció es van utilitzar per corregir la variabilitat, i aquest valor es va corregir amb el valor de tant per cent de recuperació. En el cas dels PBDEs la mitjana de recuperació de l'estàndard PCB-209 va ser 67±17%.

Programa d'intercalibratge AMAP

El protocol analític utilitzat va ser validat per l'anàlisi de materials de referència proporcionats gràcies a la participació en el programa d'intercalibratge AMAP de compostos orgànics persistents en sèrum humà (INSPQ).

Des del 1991, el *Artic Monitoring and Assessment Programme* (AMAP, 2018) ha organitzat un procés de control de sang materna a l'Àrtic, basat en estudis amb protocols estandarditzats per a la presa i l'anàlisi de les mostres. En el context d'AMAP, els laboratoris participants van expressar la necessitat d'assegurar que les dades es poguessin comparar entre elles independentment del lloc d'anàlisi. Així doncs, el 2001 es va crear un programa internacional de control d'anàlisi de diferents anàlits en sèrum. El programa està obert a tots

aquells laboratoris interessats i l'IDÆA-CSIC porta molts anys participant-hi. Tres cops a l'any els participants rebem tres mostres cada cop de materials de referència, que s'analitzen amb el mateix procediment que s'utilitza al laboratori.

Els resultats normalment estan en el marge del 20% dels valors consensuats. Això indica que són satisfactoris i que el mètode utilitzat és adequat per a l'anàlisi de compostos organohalogenats en mostres de sèrum.

Límits de detecció i quantificació

Els límits de detecció i quantificació es defineixen com:

- Límit de detecció (LD): Concentració mínima a la que es pot detectar amb fiabilitat per un anàlit.
- Límit de quantificació (LQ): Concentració mínima a la que es pot detectar l'anàlit per assegurar mesures quantitativament precises.

Normalment els compostos que queden per sota dels límits de detecció s'especifiquen com a "no detectats" (nd o <LD) i se'ls hi assigna la meitat del valor d'aquest límit. De la mateixa manera, els valors per sota dels límits de quantificació es consideren "no quantificables" (nq o <LQ), també se'ls hi assigna la meitat del valor d'aquest límit.

Tant en l'anàlisi d'OCs com de PBDEs, els LD i LQ es van calcular a partir dels blancs de procediment fent la mitjana de les concentracions observades més 3 i 5 vegades les desviacions estàndards, respectivament. Els límits de detecció variaven entre 0,0014 i 0,027 ng/mLpels OCs i entre 0,012 i 0,035 ng/mLpels PBDEs, els límits de quantificació estaven entre 0,0023 i 0,038 ng/mLper OCs i 0,017-0,040 ng/mLper PBDEs.

Anàlisi de dades

L'anàlisi de dades així com els corresponents gràfics es van dur a terme amb el programa estadístic R (R, 2018). Les anàlisis inclouen estadística descriptiva i inferència (majoritàriament regressions linears multivariants i anàlisis no paramètriques). A la secció de resultats s'indiquen les anàlisis realitzades.

Ajust per lípids

Els estudis epidemiològics normalment descriuen les concentracions d'OCs i PBDEs en mostres humanes normalitzades pel total de lípids (TL) de cada individu. La concentració de lípids en sang es pot estimar amb les concentracions de colesterol i triglicèrids segons l'equació 3.1.

$$TL = (TC * 2{,}27) + TG + 62{,}3 \qquad (3.1)$$ (Phillips et al., 1989).

TL: Lípids totals; TC: Colesterol total; TG: Triglicèrids (mg/dL)

Les concentracions de OCs i PBDEs es normalitzen individualment pel contingut total de lípids, dividint la concentració de sèrum crua (ng/mL) entre el TL. Les noves concentracions obtingudes s'expressen en nanograms d'anàlit per gram de lípid (ng/g lípid).

Normalització de les dades

Generalment, les concentracions dels compostos analitzats no segueixen una distribució normal. Per abordar aquest problema s'han adoptat dues aproximacions: l'ús de tècniques no paramètriques i la transformació logarítmica de les dades. Les anàlisis no paramètriques es van utilitzar en primer lloc, però després es van descartar a favor de la transformació logarítmica, perquè és la manera més simple de solucionar el problema de la no-normalitat. A més, la transformació logarítmica ens permet l'ús de les tècniques estàndards d'anàlisi de dades, com regressió lineal, facilitant la interpretació de les dades.

3.3 Resultats

Aquesta tesis doctoral s'ha realitzat a partir d ela publicació de diversos treballs científics. Aquest capítol resumeix els treballs publicats sobre els contaminants orgànics persistents, OCs i PBDEs, en dues poblacions de l'hemisferi sud, Salta i Ushuaia a l'Argentina i una altra població a Rússia, concretament Chukotka. Tant Ushuaia com Chukotka són poblacions remotes, la primera d'elles és una de les ciutats més properes a l'Antàrtida i la segona forma part del Cercle Polar Àrtic. Els resultats es componen dels següents estudis:

- Estudi de la influència de diverses característiques sociodemogràfiques en l'acumulació de compostos organoclorats i organobromats en dones de l'Argentina (Article 1 – publicat a la revista *Environmental Research*, 2017)
- Comparació exhaustiva entre les concentracions de compostos organoclorats trobades en les dones d'Argentina amb altres llocs del món (Article 2 – publicat a la revista *Environmental Science: Processes and impacts*, 2017)
- Estudi de l'acumulació de compostos organoclorats i organobromats en dones de l'Àrtic (Chukotka, Rússia) i l'afectació antropomètrica dels nadons acabats de néixer (Article 3 – publicat a la revista *Environmental International*, 2019).

ARTICLE 1

Influence of maternal and sociodemographic characteristics on the accumulation of organohalogen compounds in Argentinian women. The EMASAR study.

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Environmental Research 158: 750-767 (2017)

DOI 10.1016/j.envres.2017.07.033

Environmental Research 158 (2017) 759–767

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Influence of maternal and sociodemographic characteristics on the accumulation of organohalogen compounds in Argentinian women. The EMASAR study

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ARTICLE INFO

Keywords:
Organohalogen pollutants
Persistent organic pollutants
Postpartum women
Biomonitoring
Age dependence of pollutant concentrations
Parity dependence of pollutant concentrations

ABSTRACT

The occurrence of organohalogen compounds in venous serum from post-partum mothers from two Argentinian cities, Salta and Ushuaia, has been investigated (n = 698). 4,4′-DDE was the most abundant compound in these cities, with geometric means of 33 and 67 ng/g lipid weight, respectively. City of residence, age and parity were the main determinants of the accumulation of these compounds. Hexachlorobenzene (HCB) was the second most abundant pollutant in Ushuaia, 8.7 ng/g lipid, and β-hexachlorocyclohexane (β-HCH) in Salta, 7.8 ng/g lipid. Decabromodiphenyl ether was higher in Ushuaia than Salta, 8.2 and 4.1 ng/g lipid, respectively. The predominance of β-HCH, 4,4′-DDE and 4,4′-DDT in Salta was related with higher use of pesticides for agricultural applications. The observed higher concentrations of 4,4′-DDE and 4,4′-DDT in the mothers from rural + semi-urban sites than in urban areas were consistent with this agricultural origin. In addition, the most volatile organochlorine compounds included in this study, HCB and α-HCH, were mainly found in Ushuaia. The concentrations of the studied organohalogen pollutants in Argentina were lower than those found in other similar studies which is consistent with the location of these cities in the southern hemisphere.

Age, mainly for 4,4′-DDE and polychlorobiphenyl (PCB) congeners 138, 153 and 180, and parity, mainly for HCB, β-HCH, 4,4′-DDT and PCB congener 118, were the second main determinants of the concentrations of these compounds. Gestational weight gain also influenced on the maternal levels of HCB, β-HCH, 4,4′-DDT and PCB congeners 118, 138 and 153. Higher weight accumulation during pregnancy involved dilution of these persistent pollutants.

Body mass index (BMI) was a statistically significant determinant for 4,4′-DDT, α-HCH and PCB congeners 153 and 180. The observed direct correspondence between higher BMI and 4,4′-DDT concentrations was in agreement with the above reported inputs related with agricultural applications. The reverse correspondence of BMI with α-HCH and the PCB congeners indicated higher dilution at higher weight increase.

1. Introduction

Human exposure to organohalogen pollutants is a problem of public health concern due to the ubiquitous distribution, high environmental persistence and the adverse health effects of these compounds (Simonich et al., 1995; Wigle et al., 2008). Despite most of these pollutants have been banned or restricted, they are still found in environmental samples, food and human tissues (Hites, 2004; Arellano et al., 2014; Perelló et al., 2015). The chemical stability, hydrophobic properties, and lack of efficient metabolic processes for organism excretion provide these compounds with a strong bioaccumulation potential (Johnson-Restrepo et al., 2005). These aspects are particularly relevant for newborns because persistent organic pollutants (POPs) are able to cross the placenta leading to prenatal exposure of the foetus (Vizcaíno et al., 2014a; López-Espinosa et al., 2015), and infants come to life with an initial POP burden.

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http://dx.doi.org/10.1016/j.envres.2017.07.033
Received 30 March 2017; Received in revised form 15 July 2017; Accepted 17 July 2017
Available online 25 July 2017
0013-9351/

Children are more vulnerable than adults to chemical, physical, and biological hazards because they are still growing and their immune system and detoxification mechanisms are not fully developed (Olsen, 2000). Early-life exposure to POPs during pregnancy may have adverse impact on child development and health. In utero exposure has been associated with effects on foetal growth and premature delivery (Longnecker et al., 2001; Govarts et al., 2012; Casas et al., 2015; López-Espinosa et al., 2015, 2016), neurocognitive deficit (Grandjean and Landrigan, 2014; Ribas-Fitó et al., 2006; Morales et al., 2008; Costa et al., 2014; Palou-Serra et al., 2014), obesity (Valvi et al., 2012, 2014), lower respiratory tract infections and wheeze (Gascón et al., 2012; Morales et al., 2012) and hormonal disruption (Chevrier et al., 2008; López-Espinosa et al., 2009, 2016; Morales et al., 2013; Wilson et al., 2016; Llop et al., 2017). The study of these compounds in venous maternal serum near pregnancy provides significant clues on the newborn intake (Vizcaino et al., 2014a).

The most abundant POPs usually found in human tissues are hexachlorobenzene (HCB), the β-isomer of hexachlorocyclohexane (β-HCH), 4,4′-dichlorodiphenyltrichloroethane (4,4′-DDT) and its main metabolite 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE) and polychlorinated bifenils (PCBs). Polybromodiphenyl ethers (PBDEs) are also important since their concentrations are increasing both in human and environmental samples (Hites, 2004).

These compounds have been mostly synthesized and used in the northern hemisphere but their strong capacity for long-range atmospheric transport has led to a global planetary distribution (Simonich et al., 1995), including the southern hemisphere (Amin et al., 2011). However, the information available on the occurrence of these compounds in the southern hemisphere is rather limited (Wenning and Martello, 2016; Corsolini et al., 2009), particularly for what concerns human exposure.

The present study is devoted to contribute to fill this gap by analysis of maternal serum from Argentinian cohorts, representing postpartum mothers from the cities of Salta (n = 498) and Ushuaia (n = 200). The characteristics of the participant populations from these two cities are described in Økland et al. (2017). The concentrations of organochlorine compounds from these cities are compared with those in other sites in Hansen et al. (2017). The present study is devoted to elucidate the influence of age, parity, body mass index, gestational weight gain and place of residence on the body burden of these compounds.

Ushuaia is the only urban settlement in the southern coast of Tierra del Fuego Island. The local economy mostly depends on tourism, trade, and industrial development (Commendatore et al., 2012). Salta is located in North Argentina, agriculture and its related industries are important. The latitudes of these cities are very different, 54°S and 24°S, involving subpolar and subtropical climates, with daily average temperatures of 1–9 °C and 20 °C, respectively (Luchini et al., 2002). Volatile pollutants are susceptible to long-range atmospheric transport, evaporating in warm areas and condensing in cold regions (Simonich and Hites, 1995). It should be expected to find more elevated concentrations of these pollutants in Ushuaia than in Salta if the global distillation effect is a driver of their occurrence.

2. Materials and methods

2.1. Population and study design

The present work is focused in two Argentinian regions, Salta in the North and Ushuaia in the South. Maternal blood samples (n = 698) were collected randomly from April 2011 to Mars 2012 between the first and third day after delivery at the Clínica San Jorge in Ushuaia and the Hospital Público Materno Infantil in Salta Non-fasting maternal blood samples were obtained at 36 ± 12 h following delivery (median 1, range 0–3) considering that from the analytical perspective, one of the optimum sampling periods is the early postpartum days (Hansen et al., 2010). The POPs have been analysed in these samples. The study included also maternal questionnaire data and measurements of height and weight. This postpartum weight was used to obtain gestational weight gain estimates (GWG) by substraction from the reported weight prior to pregnancy plus 5 kg for child (average 3.5 kg), blood, placenta and fluid losses. This estimate differs from the standard methods (Gilmore and Redman, 2015) but no other data was available for this calculation.

The UiT The Arctic University of Norway and Stavanger University Hospital in Norway were responsible for the EMASAR study (Estudio del Medio Ambiente y la Salud Reproductiva; Study on the environment and reproductive health). Local partnerships were the private institution Clínica San Jorge in Ushuaia that is co-responsible with the public hospital for the in-hospital deliveries in the city and partly in the province, and the Hospital Público Materno Infantil in Salta that receives all the in-hospital deliveries in the city and the region. The Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC) was responsible for the chemical analyses.

The study was approved by the Ethics Committee of the Medical Association in Salta (2010/7317) and the Ministries of Health in both provinces. As required by Norwegian law, the study was then submitted to the Norwegian Regional Committee for Medical and Health Research Ethics (REC North) who also approved the study (2011/706). The study was conducted in accordance with the Helsinki declaration.

2.2. Sample preparation

Serum samples (1 mL) were placed into 10 mL centrifuge tubes and recovery standards TBB and PCB-209 were added (50–60 pg/μL). POP extraction was performed by addition of *n*-hexane (2 mL) and H_2SO_4 (3 mL), vortex mixing (1500 rpm, 30 s) and centrifugation (3500 rpm, 10 min). The supernatant *n*-hexane layer was aspirated into a second centrifuge tube using a Pasteur pipette. The acid layer was re-extracted two more times with n-hexane. All the n-hexane extracts were combined. This n-hexane solution was further purified by oxidation with 2 mL of concentrated H_2SO_4. The tubes were stirred in a vortex (1500 rpm, 90 s) and centrifuged (3500 rpm, 10 min). The acid was removed with a Pasteur pipette and more H_2SO_4 (2 mL) was added, again, which was followed by mixing and centrifuging once more. The supernatant organic phase was transferred to a conical bottomed, graduated tube and reduced to near dryness under a gentle stream of nitrogen. Then, the sample was transferred to gas chromatographic vials using three 75 μL rinses of isooctane which were then reduced to dryness under a very gentle stream of nitrogen. Finally, they were dissolved with 100 μL of PCB-142 (internal standard) in isooctane (10 pg/μL). MiliQ water (5–6 drops) was added before centrifugation when emulsions were formed (Grimalt et al., 2010).

Subsequent PBDEs analysis involved isooctane evaporation under a very gentle stream of nitrogen and dissolution with 20 μL of [3-^{13}C] BDE-209 (6.5 pg/μL) and 30 μL of BDE-118 (20 pg/μL) as internal standards (Vizcaíno et al., 2009).

2.3. Analytical procedure

Nineteen organochlorine compounds (OCs), pentachlorobenzene (PeCB), HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, PCB congeners 28, 52, 101, 118, 138, 153, 180, 2,4′-DDD, 4,4′-DDD, 2,4′-DDE, 4,4′-DDE, 2,4′-DDT and 4,4′-DDT, were quantified by gas chromatography and electron capture detection (GC-ECD, Agilent Technologies 7890A). The instrument was equipped with a HP-5MS capillary column (60 m length, 0.25 mm internal diameter, 0.25 μm film thicknesses; JW Scientific) protected with a retention gap. 2 μL were injected in splitless mode. Injector and detector temperatures were 250 °C and 320 °C, respectively. The oven temperature was held at 90°C for 2 min, increased to 130 °C at 15 °C/min and to 290 °C at 4 °C/min with a final holding time of 15 min. Ultrapure helium was used as carrier gas. Nitrogen was

the make-up gas. Compound quantification was performed as described elsewhere (Carrizo et al., 2009).

A GC (Agilent Technologies 7890N) coupled to a mass spectrometer (MS, Agilent Technologies 5975C) operating in negative chemical ionisation mode (GC-NICI-MS) was used for identification and quantification of the PBDE congeners (17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190 and 209) and for confirmation of the peak OC identification. The instrument was equipped with a low bleed fused silica capillary column (15 m length, 0.25 mm I.D., 0.10 μm film thicknesses; DB-5MS) protected with a retention gap. One μL was injected, the oven temperature was programmed from an initial temperature of 90 °C which was kept for 1.5 min followed by heating to 200 °C at 40 °C/min, a second increase up to 275 °C at 5 °C/min and a third increase to 300 °C at 40 °C/min. This temperature was held for 10 min and then increased to 310 °C at 10 °C/min with a final holding time of 2 min. Ammonia was used as reagent gas. Identification and quantification were performed by injection of PBDEs standard solutions (Vizcaíno et al., 2009).

2.4. Quality control

One procedural blank was included in each sample batch. Method detection limits were calculated from the average signals of the procedural blank levels plus three times the standard deviation. They ranged between 0.0014 and 0.027 ng/mL for the OCs and 0.012–0.098 ng/mL for the brominated compounds. The limits of quantification were calculated from the averages of the procedural blanks plus five times the standard deviation.

Method validation was made by analysis of proficiency testing materials obtained from the Arctic Monitoring and Assessment Program (AMAP Ring Test, 2014). The laboratory participates regularly in the AMAP Ring Test Proficiency Program for POPs in human serum and the results usually range within 20% of the consensus values.

2.5. Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2016). Statistics was focused on the compounds found above limit of detection in more than 30% of the samples: HCB, α-HCH, β-HCH, 4,4′-DDE, 4,4′-DDT, PCB-118, PCB-138, PCB-153, PCB-180, BDE-153, BDE-154 and BDE-209. One-half of the limits of detection and limits of quantification were assigned to non-detected and non-quantified values, respectively.

Sample serum lipid content (TL) was calculated from the cholesterol (TC) and triglyceride (Tg) concentrations (TL (g/l) = 2.27*TC + Tg + 0.623; Phillips et al., 1989).

Geometric means (GM) and 95% confidence intervals (CI) were used for descriptive analysis (Fig. 2), categorizing all the variables into groups (Table 1). Statistical differences between covariates were tested for significance using the Chi-square test (Table 1).

Before inclusion in the multivariate regression models, the compound concentrations were transformed into the natural logarithms for normalization. All variables were escalated for cross-comparison. The β coefficients and the standardized β are shown in Table 3.

These multivariate models were used to assess the effects of age, body mass index (BMI), parity and estimated GWG on the organohalogen concentrations: $\log(POP) = \beta_1(Age) + \beta_2(BMI) + \beta_3(Parity) + \beta_4(GWG) + \beta_5(Residence) + \beta_6(City) + \in$. Age, parity, body mass index and gestational weight gain were used as continuous variables. City was categorized as Salta and Ushuaia, and residence as urban (first) and semi-urban plus rural (second). Semi-urban and rural were grouped together due to the few cases.

The β coefficients were transformed into relative changes (%) for better representation (Fig. 3). For each variable, median serum concentrations by unit change (c) were calculated as $(\exp(c*\beta)-1)*100$ and the corresponding confidence intervals were calculated as $(\exp(c*\beta \pm z_{1-\alpha/2}*SE(\beta))-1)*100$, using β and standard errors (SE) from the multiregression analysis and *c* set as the difference between the first and third quartile (Barrera-Gómez et al., 2015)

In addition, generalized additive models (GAM) were performed to assess the linearity of the variables (Figs. S1–S3 in the supporting information). The R packages gmcV, visreg and ggplot were used for modelling and graphical display.

3. Results and discussion

3.1. Socio-demographic characteristics

Two hundred of the participating women were from Ushuaia and 498 from Salta (Table 1). The mean ages of the participants were 29 and 25 years, respectively, and the overall age range was between 15 and 45 years. The postpartum BMI encompassed a large spectrum of cases, from underweight (16.4 kg/m^2) to obesity (44.1 kg/m^2). In Ushuaia 79% of women were overweight or obese and this proportion was 57% in Salta. In 41% and 44% of the women from the Ushuaia and Salta, respectively, the actual newborn was the only descendant, in 36% and 24% it was the second child. Twenty-two percent and 30% of the women from these two cities had more than two children, respectively.

Only 25% of the mothers from Ushuaia and 19% from Salta met the recommendations of the Institute of Medicine (IOM) from the US National Academies of Sciences, Engineering and Medicine for GWG. These recommendations are related with the pre-pregnancy BMI of the women: normal weight: 11.25–15.75 kg, overweight: 6.75–11.25 kg and obese: 4.95–9.00 kg (Rasmussen et al., 2009). In Ushuaia half of the

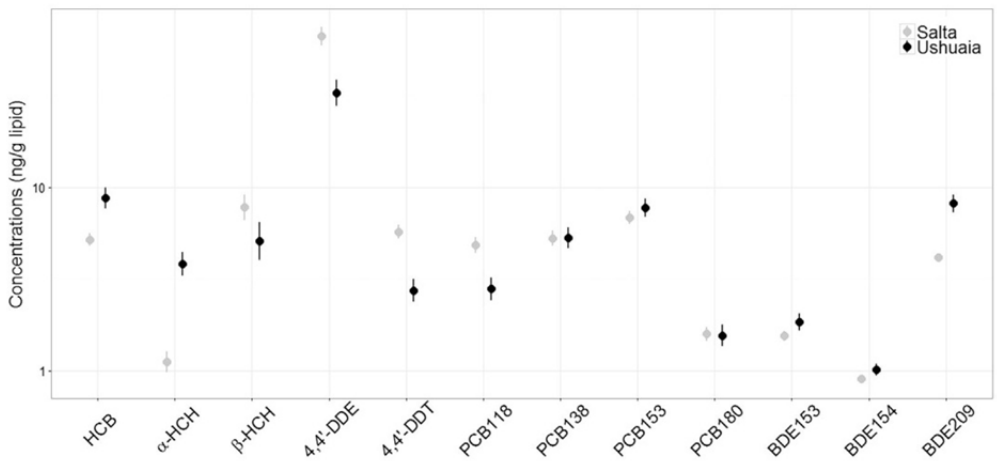

Fig. 1. Geometric means of the organohalogen concentrations in postpartum mothers from Salta and Ushuaia (ng/g lipid). The vertical bars plot the 95% confidence interval.

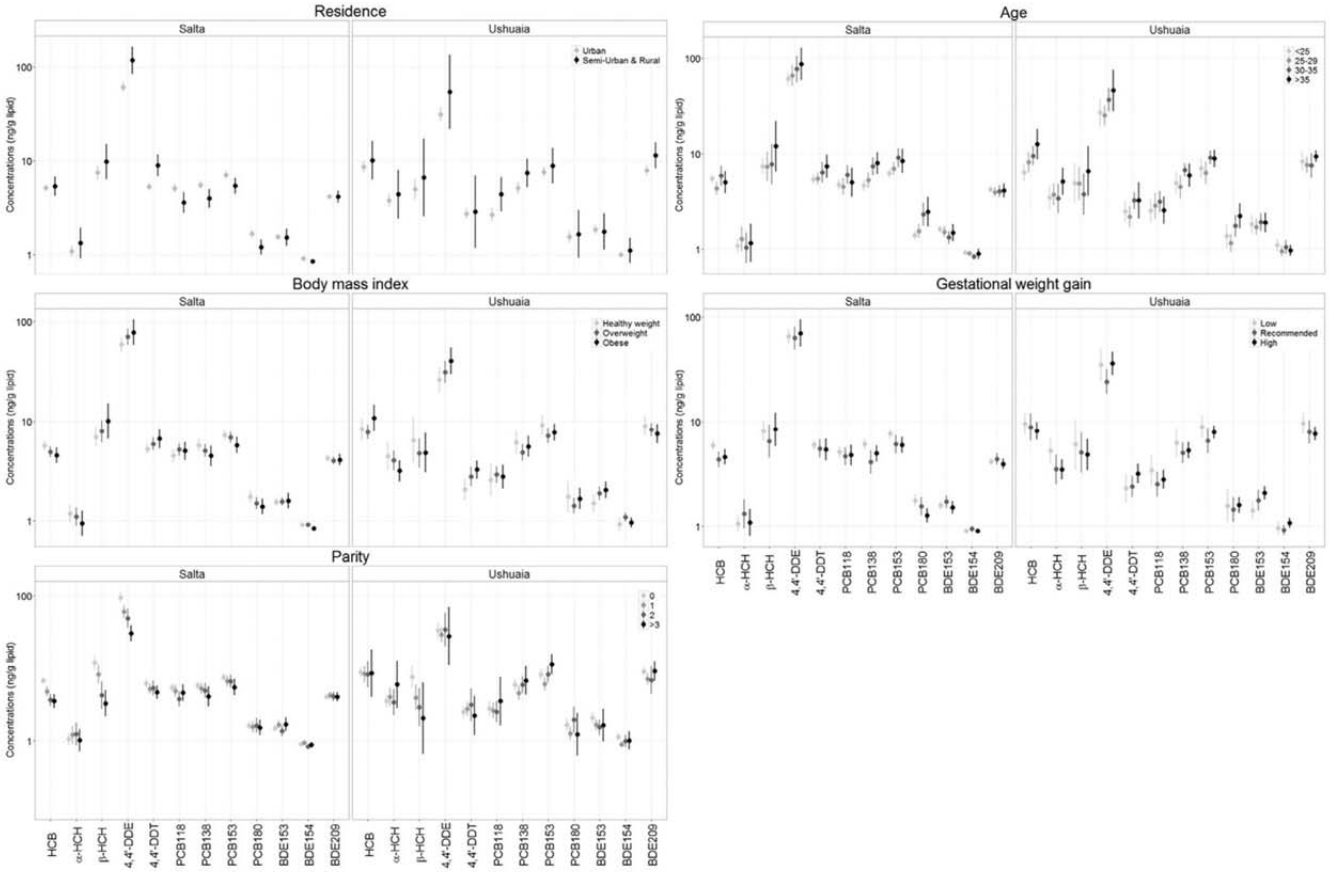

Fig. 2. Socio-demographic plots of the geometric means and the 95% confidence intervals (ng/g lipid) of the of the organohalogen compound concentrations in postpartum mothers.

Table 1
Socio-demographic characteristics of studied populations in Argentina (n = 698).

	Ushuaia – n (%)	Salta – n (%)	p-value[3]
All participants	200 (100)	498 (100)	
Age (years)			< 0.01
< 25	53 (27)	278 (56)	
25–29	56 (29)	111 (22)	
30–34	46 (24)	58 (12)	
≥ 35	40 (20)	48 (10)	
Postpartum body mass index			< 0.01
Normal weight (< 25 kg/m^2)	41 (20)	219 (45)	
Overweight (25–30 kg/m^2)	99 (50)	181 (37)	
Obese (≥ 30 kg/m^2)	60 (30)	89 (18)	
Parity[1]			< 0.01
1	82 (41)	221 (44)	
2	75 (37)	120 (24)	
3	33 (17)	78 (16)	
≥4	10 (5)	78 (16)	
Gestational weight gain[2]			< 0.01
Low	17 (9)	157 (36)	
Recommended	25 (13)	104 (24)	
High	150 (78)	180 (41)	
Residence			0.091
Urban	183 (91)	429 (86)	
Semi-urban and rural	15 (8)	36 (7)	

[1] Current alive children + stillborn after week 23.
[2] GWG groups are based on the IOM recommendations.
[3] p-value from $\times^2$ (Chi-square) *t*-test.

participants had a high GWG, while GWG was low in half of the participants from Salta.

Almost all participants lived in urban areas, 91% in Ushuaia and 86% in Salta. The 9% and 13% lived in semi-urban or rural zones while just 2 participants were from an industrial site. Concerning educational level, 48% of the participants had tertiary or university studies in Ushuaia while this group was 10% in Salta.

3.2. Organohalogen compound distributions

4,4′-DDE was found above limit of detection in almost 100% of the samples. PCB congeners 138 and 153 and 4,4′-DDT were found above limit of detection in about 90–97% of the mothers. In Ushuaia, α-HCH and HCB, 87% and 81%, respectively, were the following most abundant compounds, while in Salta the following most abundant were PCB congener 118 and β -HCH (79% and 70%, respectively). Most of the compounds were found above the limit of detection in around 50–70% and the remaining pollutants were only found in less than 48% of the serum samples (Table S1). The principal source of exposure to these compounds among the general population is diet. They are found mainly in animal products, including meat, fish, dairy products and eggs (Junqué et al., 2017; Llobet et al., 2003; Martí-Cid et al., 2007).

Only four of the 14 PBDEs were above limit of detection in 30% of the samples. The BDE congener found in most cases was 209, 93% in Ushuaia and 42% in Salta, followed by 153, 154 and 47 (20–53%) (Table S1). In the indoor environment, these compounds are associated to dust ingestion, both at home and in the workplace (Jones-Otazo et al., 2005). Children, specifically, tend to accumulate them.

The most abundant OC in both cities was 4,4′-DDE, with GM of 33 ng/g lipid and 67 ng/g lipid in Ushuaia and Salta, respectively (Table 2, Fig. 1), followed by HCB (8.7 ng/g lipid) in Ushuaia and β-HCH (7.8 ng/g lipid) in Salta. β-HCH was the most dominant HCH isomer while PCB-153 was the most abundant PCB congener in the mothers of both cities, followed by PCB-138 and PCB-118.

The most abundant BDE congener was 209, 8.2 ng/g lipid and

Table 2
Serum POP concentrations (ng/g lipid) in the population of study.

	Ushuaia (n = 199)								Salta (n = 471)							
	% > LOD[a]	GM (95%CI)[b]		Min	50th	90th	Max	50th[c]	% > LOD[a]	GM (95%CI)[b]		Min	50th	90th	Max	50th[c]
HCB	81	8.7	(7.6–10)	1.2	8.3	25	448	0.067	58	5.2	(4.8–5.6)	1.2	5.8	15	86	0.043
α-HCH	87	3.9	(3.3–4.4)	0.39	4.1	14	56	0.032	32	1.1	(0.99–1.3)	0.25	0.51	14	38	0.0030
β-HCH	62	5.1	(4.0–6.5)	0.40	6.8	48	258	0.57	70	7.8	(6.6–9.1)	0.42	11	65	408	0.074
4,4′-DDE	99	33	(28–39)	0.89	27	146	20,547	0.22	100	67	(59–75)	2.9	58	371	10,677	0.42
4,4′-DDT	90	2.7	(2.4–3.2)	0.25	3.0	7.7	1682	0.022	97	5.7	(5.2–6.2)	0.30	5.2	17	286	0.039
PCB-118	65	2.8	(2.4–3.2)	0.37	3.3	11	53	0.025	79	4.8	(4.4–5.3)	0.47	6.1	15	139	0.042
PCB-138	97	5.3	(4.6–6.1)	0.094	5.8	14	37	0.046	97	5.3	(4.8–5.8)	0.12	5.9	18	146	0.041
PCB-153	97	7.7	(6.9–8.7)	0.40	8.1	20	40	0.064	94	6.8	(6.3–7.4)	0.38	7.3	20	123	0.051
PCB-180	37	1.6	(1.4–1.8)	0.29	1.0	6.3	29	0.0055	30	1.6	(1.5–1.7)	0.51	1.0	7.8	53	0.0060
BDE-153	75	1.8	(1.7–2.1)	0.31	1.9	4.4	38	0.017	58	1.6	(1.5–1.7)	0.52	1.2	3.8	71	0.0083
BDE-154	35	1.0	(0.95–1.1)	0.41	0.87	2.5	6.6	0.0060	30	0.91	(0.88–0.93)	0.51	0.86	1.2	3.8	0.0060
BDE-209	92	8.2	(7.3–9.1)	0.65	2.8	15	147	0.068	92	4.1	(3.9–4.4)	1.5	3.5	9.2	27	0.020

[a] % of samples above the limit of detection.
[b] GM(95%CI): Geometric mean with 95% confidence intervals.
[c] Median in ng/mL.

Table 3
Results of the regression models showing effects of various determinants in blood serum (n = 599).

Compound	Variable	β[a,b]	Std. β[b]	P	Compound	Variable	β[a]	Std. β[b]	P
HCB	Age	0.034	0.24	p < 0.001	**PCB-138**	Age	0.056	0.36	p < 0.001
	BMI[1]	0.0095	0.044	0.30		BMI[1]	− 0.015	− 0.062	0.15
	Parity	− 0.27	− 0.39	p < 0.001		Parity	− 0.23	− 0.30	p < 0.001
	GWG[2]	− 0.020	− 0.16	p < 0.001		GWG[2]	− 0.015	− 0.11	p < 0.05
	City[3]	0.38	0.19	p < 0.001		City[3]	− 0.15	− 0.065	0.12
	Residence[4]	0.14	0.049	0.21		Residence[4]	− 0.13	− 0.041	0.29
α-HCH	Age	0.012	0.056	0.27	**PCB-153**	Age	0.046	0.34	p < 0.001
	BMI[1]	− 0.032	− 0.092	p < 0.05		BMI[1]	− 0.019	− 0.094	p < 0.05
	Parity	− 0.0021	− 0.0019	0.97		Parity	− 0.19	− 0.29	p < 0.001
	GWG[2]	0.012	0.064	0.14		GWG[2]	− 0.011	− 0.096	p < 0.05
	City[3]	1.2	0.37	p < 0.001		City[3]	− 0.034	− 0.018	0.68
	Residence[4]	0.25	0.056	0.16		Residence[4]	− 0.18	− 0.067	0.098
β-HCH	Age	0.078	0.29	p < 0.001	**PCB-180**	Age	0.061	0.42	p < 0.001
	BMI[1]	0.037	0.090	p < 0.05		BMI[1]	− 0.027	− 0.12	p < 0.01
	Parity	− 0.61	− 0.46	p < 0.001		Parity	− 0.19	− 0.27	p < 0.001
	GWG[2]	− 0.025	− 0.11	p < 0.05		GWG[2]	− 0.0076	− 0.061	0.18
	City[3]	− 0.83	− 0.21	p < 0.001		City[3]	− 0.026	− 0.012	0.087
	Residence[4]	0.41	0.075	0.058		Residence[4]	− 0.29	− 0.10	p < 0.05
4,4′-DDE	Age	0.071	0.36	p < 0.001	**PBDE-153**	Age	− 0.011	− 0.10	0.069
	BMI[1]	0.027	0.089	P < 0.05		BMI[1]	0.0059	0.035	0.46
	Parity	− 0.46	− 0.47	p < 0.001		Parity	0.030	0.056	0.29
	GWG[2]	− 0.012	− 0.071	0.085		GWG[2]	− 0.012	− 0.012	0.79
	City[3]	− 1.1	− 0.38	p < 0.001		City[3]	0.18	0.11	p < 0.05
	Residence[4]	0.59	0.15	p < 0.001		Residence[4]	− 0.047	− 0.021	0.62
4,4′-DDT	Age	0.023	0.15	p < 0.01	**PBDE-154**	Age	− 0.0040	− 0.072	0.19
	BMI[1]	0.027	0.11	p < 0.01		BMI[1]	− 0.0039	− 0.045	0.34
	Parity	− 0.12	− 0.15	p < 0.01		Parity	0.0015	0.0054	0.92
	GWG[2]	− 0.014	− 0.11	p < 0.05		GWG[2]	0.0031	0.063	0.18
	City[3]	− 0.83	− 0.37	p < 0.001		City[3]	0.11	0.14	p < 0.01
	Residence[4]	0.36	0.11	p < 0.01		Residence[4]	− 0.015	− 0.013	0.76
PCB− 118	Age	0.022	0.13	p < 0.01	**PBDE-209**	Age	0.00013	0.0011	0.98
	BMI[1]	0.018	0.072	0.11		BMI[1]	− 0.0088	− 0.051	0.23
	Parity	− 0.15	− 0.19	p < 0.001		Parity	0.018	0.034	0.48
	GWG[2]	− 0.021	− 0.15	p < 0.01		GWG[2]	0.00082	0.0085	0.84
	City[3]	− 0.60	− 0.25	p < 0.001		City[3]	0.68	0.43	p < 0.001
	Residence[4]	− 0.12	− 0.037	0.37		Residence[4]	0.12	0.054	0.17

[a] β Coefficients of the multivariate regression models with non-standardized variables.
[b] β coefficients of the multivariate regression models after standardizing all the variables.
[1] BMI: Body mass index.
[2] GWG: Gestational weight gain.
[3] Salta as the reference city.
[4] Urban as reference category for residence.

4.1 ng/g lipid in Ushuaia and Salta, respectively, followed by 138, 153 and 154 (Table 2).

The concentrations of β-HCH, 4,4′-DDE, 4,4′-DDT and PCB congener 118 were significantly higher in Salta than in Ushuaia ($p < 0.001$; Table 3 and Fig. 1). The concentrations of DDT compounds in the mothers from the former city were two times higher than those in the second (Table 2, Fig. 1) which may reflect much stronger use of organochlorine pesticides in relation to past agricultural activities. Conversely, the mothers from Ushaia showed significant higher concentrations of HCB ($p < 0.001$), α-HCH ($p < 0.001$), BDE congeners

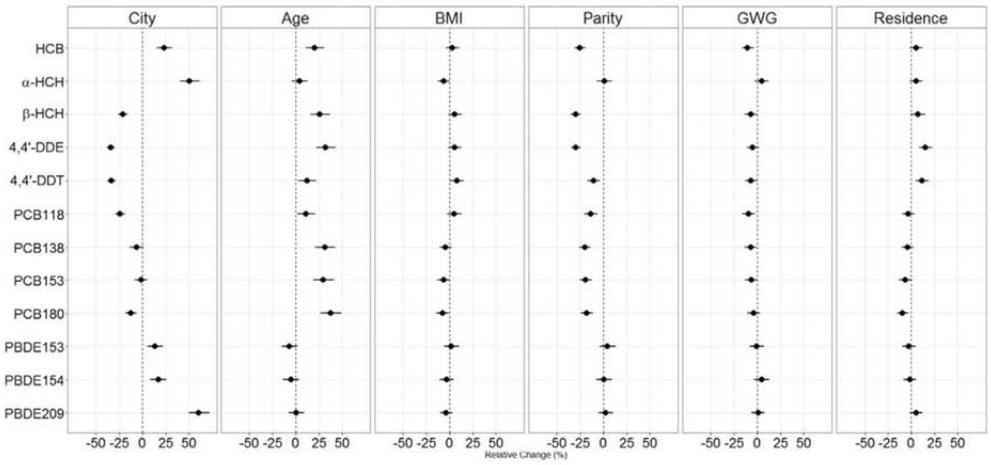

Fig. 3. Relative change (%) in median serum organohalogen concentrations by unit change calculated from the β coefficients and standard errors of the multi-regression analysis described in Table 3. The units of changes for each variable were set as the difference between the first and third quartile.

153 ($p < 0.05$), 154 ($p < 0.01$) and 209 ($p < 0.001$). The concentrations of α-HCH and BDE congener 209 in the former were two times or higher than those in the latter (Table 2, Fig. 2). The most volatile compounds in this study, HCB and α-HCH, were mainly found in Ushuaia (Fig. 1).

City of stay was the only determinant which significant influenced on the concentrations of the BDE congeners which were higher in Ushuaia than in Salta (Table 3). This difference suggested a higher use of furniture, computers and other recently-made material treated with PBDE as flame retardant in the former than in the latter city. The difference was also consistent with the higher proportion of participants with tertiary or university studies in Ushuaia than Salta (Table 1).

Other compounds such as most PCB congeners did not show significant differences between the two cities (Table 3, Fig. 1).

Compared to other similar studies, these Argentinean postpartum women have low levels of the analysed compounds. The concentrations of 4,4′-DDE in Ushuaia were similar to those found in Norway (Hansen et al., 2010) or Brazil (Rudge et al., 2012), slightly lower than those from Salta and much lower than the levels from Asturias (Vizcaíno et al., 2014a). Sum of the studied PCBs were found to be much lower in the present study than in all the above cited sites and Bolivia (Arrebola et al., 2016). Finally, HCB showed similar concentrations in Ushuaia and Norway (Hansen et al., 2010), both polar regions with comparable climate and dietary habits. These differences are consistent with the location of these cities in the southern hemisphere in which organochlorine compounds were much less used. A more extensive comparison between the OC concentrations in the studied cohorts of these two cities and others from other geographic areas is provided in Hansen et al. (2017).

3.3. Residence

Comparison of the maternal concentrations by residence showed significant differences in Salta (Fig. 2). Higher levels were observed in the mothers living in semi-urban and rural sites than in urban areas. This difference was consistent with a high use of DDT in agriculture. Higher maternal DDE concentrations in semi-urban and rural areas than in urban sites were also observed in Ushuaia although the difference was not significant. In this case, the lack of significance was due to the high dispersion of the values in the semi-urban and rural group likely as consequence of the low number of individuals ($n = 17$, Table 1). In any case, the 4,4′-DDE and 4,4′-DDT concentrations in Salta was consistent with higher agricultural activities than in Ushuaia.

The maternal PCB concentrations in Salta were significantly higher in the urban group than in the combined semi-urban and rural groups (Fig. 2) which suggested higher exposure to PCB contamination in the urban environment of this city.

3.4. Age

In general, the maternal concentrations of the OCs showed a positive significant correlation with age (Table 3). Old women tended to have higher levels than younger women (Fig. 2). The differences were particularly significant in Salta for the PCB congeners 138, 153 and 180 and in Ushuaia for HCB and PCB congener 180 (Fig. 3). Increases of the concentrations of PCBs and organochlorine pesticides with age have been observed in general population (Porta et al., 2010; Nøst et al., 2013) and maternal cohorts, e.g. cord blood (Carrizo et al., 2006; Vizcaino et al., 2010; Hansen et al., 2010; Veyhe et al., 2015)

No age dependence (Antignac et al., 2009; Jin et al., 2009; Zota et al., 2008) or higher concentrations in the young population (Garí and Grimalt, 2013) have been observed for PBDEs. In the present case, higher concentrations are observed in the younger mothers (Fig. 2 and Table 3) but the differences are not significant, probably because of the short age interval of the participating individuals.

3.5. Parity

Parity records included the cases of stillbirth after week 23. Higher values were associated with significantly lower concentrations of all OCs except α-HCH (Table 3). This trend was clearly observed in Salta and to a lower extent in Ushuaia. (Fig. 2). Parity has been found to be inversely correlated with plasma POPs (Polder et al., 2009; Hansen et al., 2010; Veyhe et al., 2015), breast milk concentrations (Manaca et al., 2011) and cord blood serum (Manaca et al., 2013), since delivery provides a way of eliminating part of the burden of these pollutants.

3.6. Body mass index

Significant correlations between BMI and the concentrations of some OCs were found (Table 3). However, they had different sign. While higher BMI involved higher maternal 4,4′-DDT concentrations, they corresponded to lower concentrations of α-HCH and PCB congeners 153 and 180 (Fig. 2). These differential trends have been observed in the other studies. For instance, in serum from women of the Child Health and Development Study Cohort in the San Francisco Bay Area of California, PCB and 4,4′-DDE decreased with increasing BMI but heptachlor epoxide and 2,4′-DDT and 4,4′-DDT rose at higher BMI (James et al., 2002). In serum from a representative sample of the population of Catalonia most OCs increased at higher BMI but PCB congeners 153 and 180 decreased (Porta et al., 2010). In cord blood serum from a cohort in Menorca Island significantly higher concentrations of HCB and 4,4′-DDE were observed at higher BMI but no significant correlations were observed for the other compounds (Carrizo et al., 2006). In Germany, a study of breast milk found a negative

relationship between BMI and lipid-adjusted PCBs but not with pesticides (Schade et al., 1998).

These discrepant correlations may reflect the pollutant composition of the predominant food sources in the studied cohorts. Higher BMI involves higher fat body burden. When POPs are absent or in very low amounts in the food sources higher BMI may involve tissue dilution and lower serum concentrations. On the contrary, POPs will tend to bioaccumulate in body tissues and higher BMI will involve higher serum concentrations. In the present study, the compounds showing direct correlations with BMI were 4,4′-DDE and 4,4′-DDT (Fig. 2) which were those related with agricultural activities. In contrast, the reverse correlations of α-HCH and PCB congeners 153 and 180 may reflect past exposures but low current food pollutant concentrations.

No significant correlations between the maternal serum PBDE concentrations and BMI were found in the studied Argentinian cohorts (Table 3). Similarly, BMI was not a significant determinant of the concentrations of these pollutants in adult population from a representative Catalan cohort (Garí and Grimalt, 2013) or postmenopausal women of Quebec (Sandanger et al., 2007). However, lower serum concentrations of BDE-153 in obese elderly Swedish fishermen's wives were found at higher BMI (Weiss et al., 2006) and higher PBDE concentrations at higher BMI in cohorts of US consumers of sport-caught fish (Anderson et al., 2008) and pregnant women from Monterrey County (California, USA, Chamacos cohort, Castorina et al., 2011) were observed.

3.7. Gestational weight gain

The distribution of total GWG estimated was grouped according to the IOM recommendations as low, recommended and high. Since the method of estimation of GWG used in this study was using post-partum weight, grouping by the IOM recommendations could have more dispersion errors than when using standard methods (Gilmore and Redman, 2015). However, the observed dependences of the concentrations of some compounds were significant and showed clear trends. Thus, the observed data could be interpreted to indicate a clear dependence between concentrations of some pollutants and GWG of the women from this cohort which would be better defined if this weight parameter had been calculated following a more standard procedure. HCB and PCB congeners 118, 138 and 153 showed substantially higher serum concentrations in the mothers whose GMG was low (Fig. 2). This difference was not observed for the DDTs or PBDEs. These results were consistent with a previous study of Swedish pregnant women in which inverse relations between GWG and maternal serum concentrations of PCB congeners 118, 138, 153, 156 and 180 and HCB were found (Glynn et al., 2007) as well as for PCB congeners in mothers from Michigan and Texas (Jaacks et al., 2016) and in the Norwegian Mother and Child cohort study (Caspersen et al., 2016). This inverse relationship has also been observed when comparing GWG and cord blood (Vizcaino et al., 2014a, 2014b).

3.8. Multiregression analysis

Linear and non-linear multivariate models of the aforementioned variables (Table 3, Fig. 3) provided an overall description of the main factors influencing on the concentrations of these organohalogen pollutants. City of residence was the main determinant, involving higher concentrations of HCB, α-HCH and PBDEs in Ushuaia and higher concentrations of β-HCH, 4,4′-DDE, 4,4′-DDT and PCB congeners 118 and 180 in Salta. This influence is consistent with a higher use of DDT and also β-HCH related with agricultural applications in the latter and a more urban life style involving higher use of PBDEs in the former. Age was the main second determinant of 4,4′-DDE and PCB congeners 138, 153 and 180 (Fig. 3). Parity was the main second determinant of HCB, β-HCH, 4,4′-DDT and PCB congener 118 (Fig. 3). These two determinants alternative scored as the third cause of change when the other was the second. Thus, city of residence, age and parity were clear determinants of the concentrations of these pollutants, although PBDE concentrations were only influenced by the former.

GWG was observed to be a fourth significant factor of variation which was inversely related to the maternal serum concentrations of HCB, β-HCH, 4,4′-DDT and PCB congeners 118, 138 and 153. BMI was also a determinant for PCB congeners 153 and 180, α-HCH and 4,4′-DDT.

Urban vs rural + semi-urban residence was relevant for 4,4′-DDE and 4,4′-DDT which is consistent with the above mentioned influence of agricultural uses of these compounds.

4. Conclusions

City of residence, age and parity are the main aspects determining the accumulation of OCs in the studied cohorts. The former reflects a higher use of DDT and also β-HCH related with agricultural activities in Salta and higher use of furniture and electronics treated with flame retardants in Ushuaia. Age is involving higher concentrations of the OCs when it is significantly related with the maternal accumulation of these pollutants which is consistent with difficulties of human metabolism to eliminate these compounds after intake. Parity is inversely related to the concentrations of OCs when it is a statistically significant determinant which reflects a clean detoxification of the mother into the newborns. Urban vs rural + semi-urban residence was relevant for 4,4′-DDE and 4,4′-DDT which is consistent with the above mentioned influence of agricultural uses of these compounds.

Women with low GWG had significantly higher concentrations of HCB and some PCB congeners. This determinant has a lower level of relevance for OC accumulation than those previously described. However, it is inversely related to the maternal concentrations of the OCs for all the compounds in which it is statistically significant. Higher accumulation of weight during pregnancy involves dilution of these persistent pollutants.

BMI was a statistically significant determinant for 4,4′-DDT, α-HCH and PCB congeners 153 and 180. The direct correspondence between higher BMI and the concentrations of 4,4′-DDT is in agreement with the above reported inputs of this compound related with agricultural applications. Since POPs tend to bioaccumulate in body tissues the presence of these compounds in food involves higher serum concentrations at higher BMI. The reverse correspondence of BMI with α-HCH and the PCB congeners indicate higher dilution at higher weight gain when the food incorporation of these compounds is low.

The most volatile organochlorine compounds included in the study, HCB and α-HCH, were found in higher concentration in the colder area (Ushuaia).

Acknowledgements

The authors acknowledge the Health Ministries of the provinces Salta and Tierra del Fuego, Argentina for their administrative support and facilitation. We acknowledge Dr. Martin de la Arena for his assistance in obtaining the ethics approval and initiating the study in Argentina, Bente A. Augdal for designing the biological sampling procedures and managing the study biobank in Norway, Dr. Silvia dib Ashur for implementation of the routines for biological sampling and Lic. Maria José Aleman (Salta) and Maria Florence Bressan (Ushuaia) for their key roles in the local administration of the study. The authors also wish to thank the Norwegian Ministry of Foreign Affairs for partial funding through the Arctic Monitoring and Assessment Programme (AMAP). We also acknowledge financial support from the EU projects NEUROSOME (H2020-MSCA-ITN-2017-766251) and HEALS (FP7-ENV-2013-603946) and project GRACCIE_REDES (CTM2014-59111-REDC).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.07.033.

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MATERIAL SUPLEMENTARI

Influence of maternal and sociodemographic characteristics on the accumulation of organohalogen compounds in Argentinian women. The EMASAR study.

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Figure S1. GAM plots for OCPs.

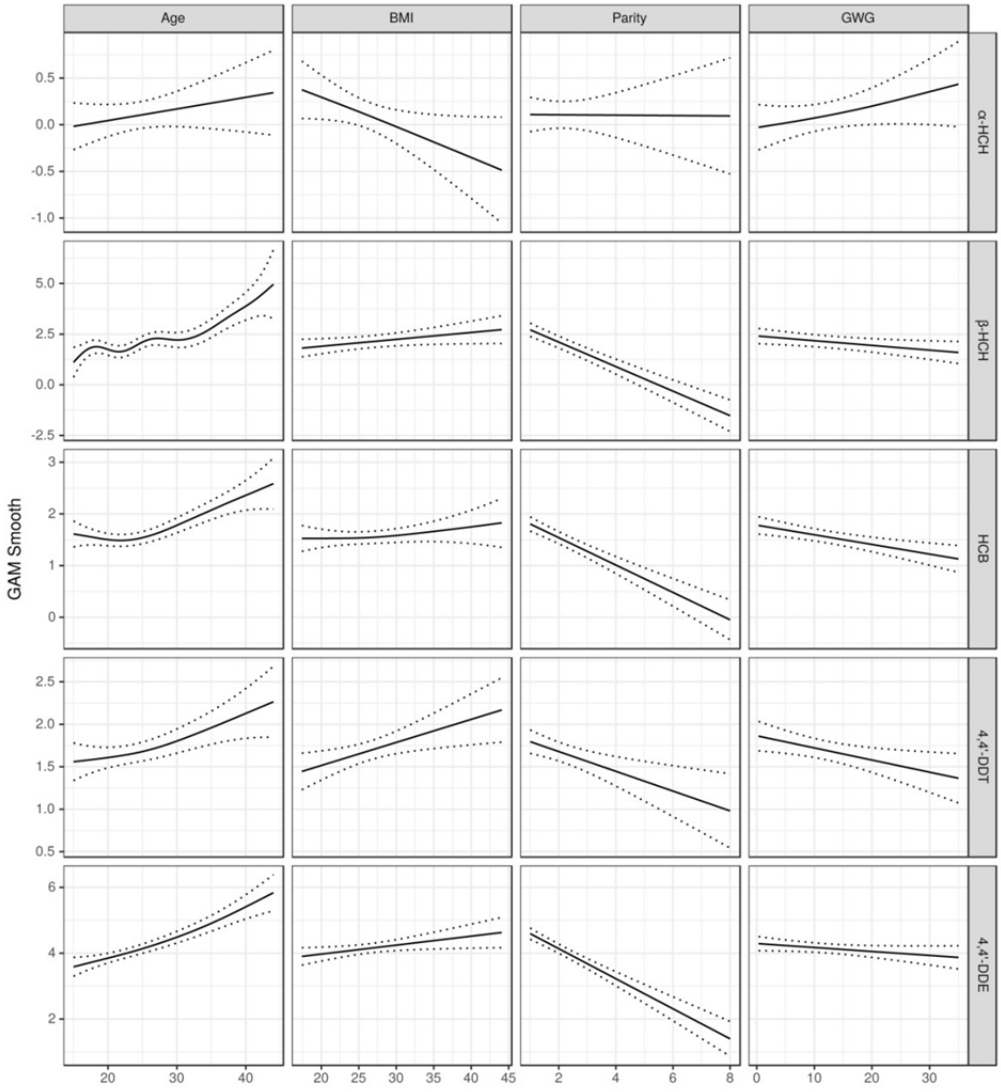


Figure S2. GAM plots for PCBs.

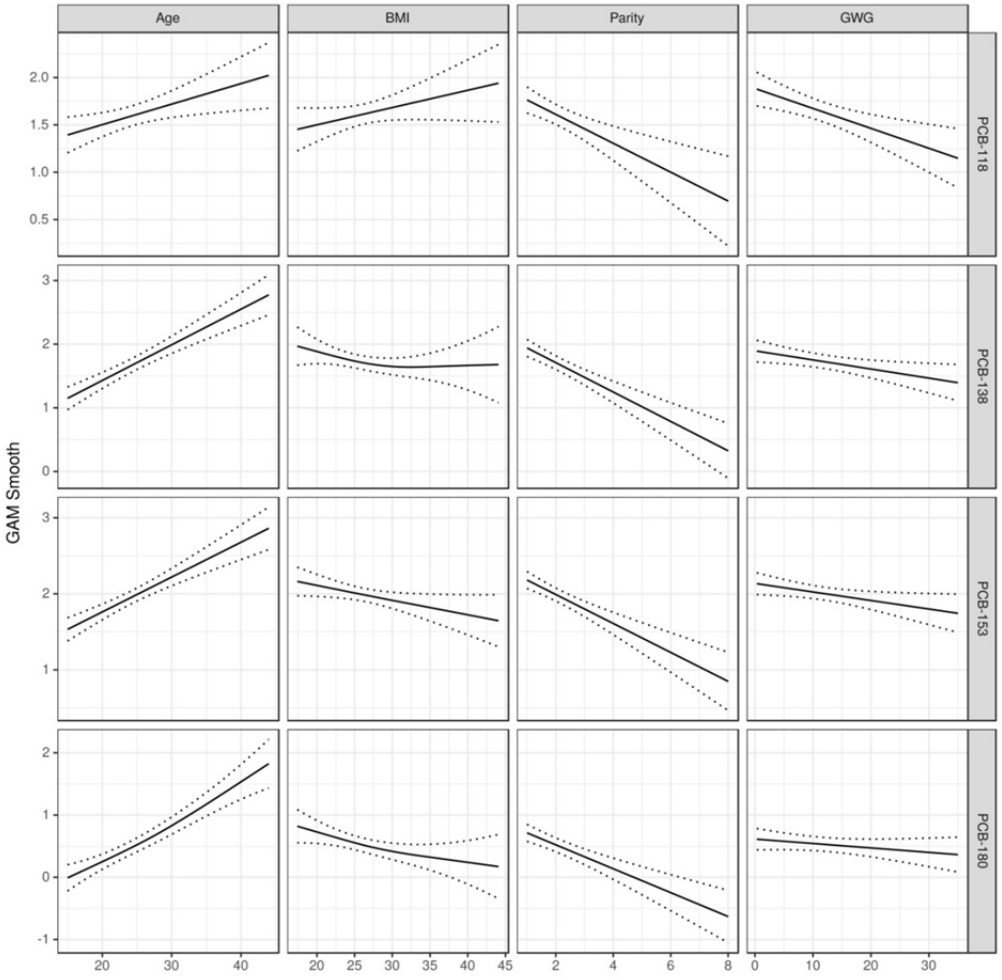
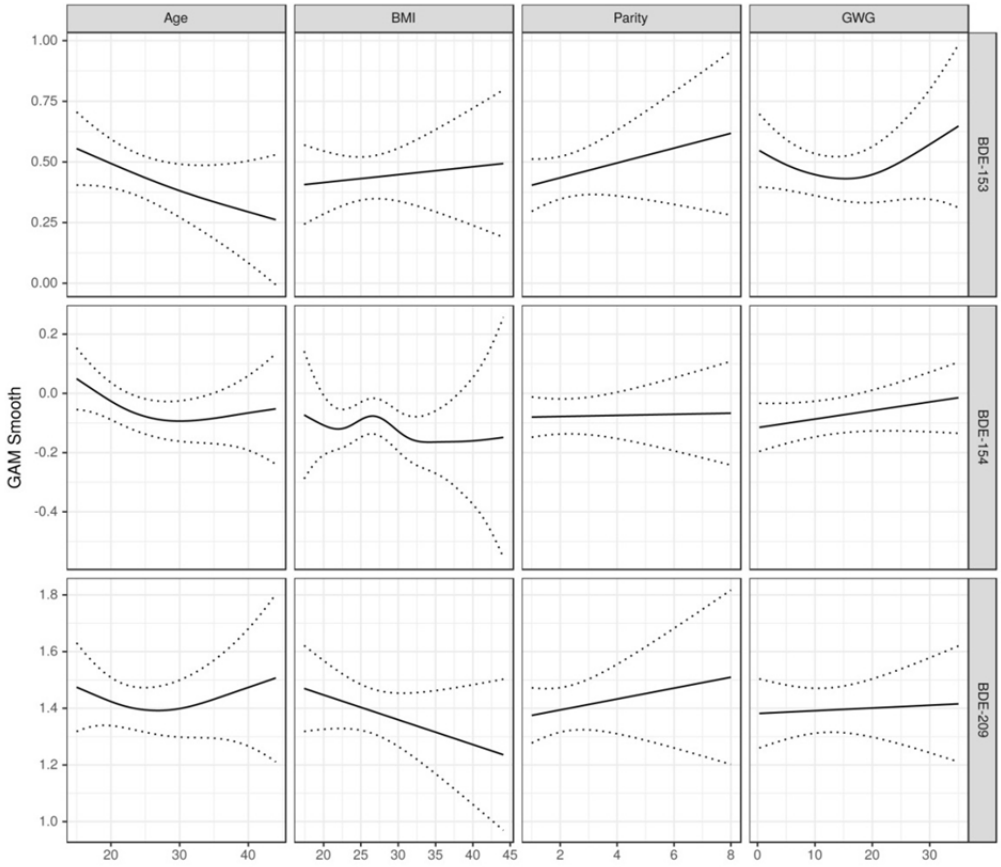


Figure S3. GAM plots for PBDEs.



ARTICLE 2

Variations in serum cocentrations of selected organochloines among delivering women in Argentina. The EMASAR study.

Solrunn Hansen, Evert Nieboer, Natalia Bravo, Inger Økland, Silvina Matiocevich, Marisa V. Álvarez, Joan O. Grimalt i Jon-Øyvind Odland

Environmental Science: Processes & Impacts 19: 1542-1553 (2017)

DOI 10.1039/c7em00278e

Environmental Science
Processes & Impacts

PAPER

View Article Online
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Cite this: *Environ. Sci.: Processes Impacts*, 2017, **19**, 1542

Variations in serum concentrations of selected organochlorines among delivering women in Argentina. The EMASAR study†

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Received 19th June 2017
Accepted 27th September 2017

DOI: 10.1039/c7em00278e

rsc.li/espi

The EMASAR study is the first study to describe the body burden of OCs in Argentinian women after delivery. In total, 698 maternal serum samples from Salta ($n = 498$) and Ushuaia ($n = 200$) were collected in 2011–2012 and analyzed for a total of 7 polychlorinated biphenyls (PCBs) and 12 pesticide-related compounds. Only 11 of the compounds had detection rates above 60% in one or both places. Compared with Ushuaian women, those from Salta exhibited higher lipid-adjusted concentrations of *p,p′*-DDE, *p,p′*-DDT, β-HCH, and PCB 118 ($p \leq 0.003$), with no differences in concentrations of PCB 153 and 138. After controlling for age, parity and heritage (born in the province or migrated there from other regions of Argentina), concentrations of *p,p′*-DDE, *p,p′*-DDT, β-HCH and all PCBs were significantly higher in Salta natives compared with Ushuaia natives or migrants ($p \leq 0.010$). No variations between native and migrated Ushuaian women were observed other than for PCB 153 (6.1 *versus* 8.6 μg kg^{-1} lipid, $p = 0.022$). Age was generally associated positively with the body burden of nearly all OCs and parity negatively so, with *p,p′*-DDD, *o,p′*-DDT, and *o,p′*-DDD residues and α-HCH in Ushuaia being the exceptions. The regional differences in OC concentrations are explained by contrasting domestic sources, historical and current uses, industrial emissions, dietary patterns and lifestyle factors, as well as long-range-transport. The relatively high PCB 118/PCB 180 ratio observed for both Argentinian communities likely reflects the use of technical mixtures with congener-specific composition. In a comprehensive comparison with other countries, the Argentinian OC concentrations were mostly in the lower range. It is concluded that a latitude effect equivalent to that operative in the Arctic region seems unlikely.

Environmental significance

Knowledge about human exposure to organochlorine compounds (OCs) in Southern Hemisphere countries is limited. Polychlorinated biphenyls (PCBs) and pesticides in the sera of delivering women in Salta (a northwestern province) and Ushuaia (the most southern province) of Argentina reveal regional differences in concentrations and these are explained by contrasting domestic sources, historical and current uses, industrial emissions, dietary patterns, lifestyle factors and long-range-transport. A comprehensive comparison with other countries indicates that the Argentinian OC concentrations were mostly in the lower range.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7em00278e

1. Introduction

Most OCs‡ were synthesized and used in the Northern Hemisphere.[1,2] Furthermore, the Arctic has become a repository for contaminants due to what is referred to as the latitude effect that involves atmospheric transportation and ocean currents.[3] As a consequence, most studies of the body burdens of OCs in humans have been conducted among Northern Hemisphere populations.[3] Comparable information for southern-latitude countries is limited. The Southern American continent constitutes a potential sink for OCs from global sources.[4] Environmental studies of air, sediments, soil, water, wildlife and biota have indeed regarded their occurrence there as a consequence

‡ Full names of the abbreviations used are provided in Section 2.2.

of both global transport and past local uses.[5–9] DDT and HCHs have been used in agriculture, while PCBs have been linked to inputs from power plants, industrial activities, combustion and dumping.[5,6,9] Not surprisingly, these pollutants have entered the domestic food web.[4,6,10,11] Argentina ratified the Stockholm Convention that banned DDT, HCB and HCHs in agriculture uses since the late 1990s, as well as placing restrictions or bans on PCBs in 2001 (ref. 4) and more recently on the use of the insecticide dicofol.[4,9,10]

OCs are persistent, lipophilic, toxic and bioaccumulate in the food chain.[3] The concentration of contaminants in maternal blood and breast milk provides important information on the exposure of the fetus and newborn, who are especially vulnerable. Endocrine disruption, reproductive effects, and impairment of immunologic development constitute worries and their adverse toxic effects may threaten human health (including *trans*-generational impairments).[3]

Knowledge about human exposure to OCs in South America is limited.[12] The current study describes the body burden of OCs in Argentinian delivering women, and our findings are assessed in the context of comparable studies elsewhere. Our working hypothesis is that a phenomenon similar to the Arctic latitude-transport effect is not operative in the Southern Hemisphere.

2. Materials and methods

2.1 Description of the study area and data collection

The EMASAR study was conducted in the Argentinian cities of Ushuaia and Salta (for map see Fig. 1 in Økland *et al.* 2017).[13] Ushuaia (54.80° S, 68.30° W) is the southernmost city in the world and the capital of the Tierra del Fuego in Antártida e Islas del Atlántico Sur Province. It has a population of some 60 000 and features electronics manufacturing, fisheries, natural gas and oil extraction, sheep farming, and tourism. Salta (24.78° S, 65.42° S) is the capital of the northwestern-highland Salta province. The city has around 620 000 citizens, with agriculture and related industrial activities being the main economic activities (for additional details see Økland *et al.*, 2017).[13]

The field work was conducted in the period April 2011 to March 2012. Of the total 698 delivering women enrolled, the current study component is limited to 670 subjects (199 from Ushuaia and 471 from Salta). One person from Ushuaia and 27 from Salta were excluded due to the lack of serum lipid concentrations. Through interviews, participants completed questionnaires covering personal characteristics, socioeconomic factors, obstetrical and breastfeeding history, environmental, health and lifestyle conditions, and dietary intake. Non-fasting maternal blood samples, height and weight were obtained at 36 ± 12 hours following delivery (median 1 day, range 0–3 days). Details about the study profile, population anthropometric measurements and blood sampling procedures have been provided previously.[13]

EMASAR is a collaborative project between UiT The Arctic University of Norway, the Stavanger University Hospital (Norway) and the two Argentinian partners, namely Clínica San Jorge in Ushuaia (a private institution co-responsible with a public hospital for the in-hospital deliveries in the city and the surrounding region), and the Hospital Público Materno Infantil

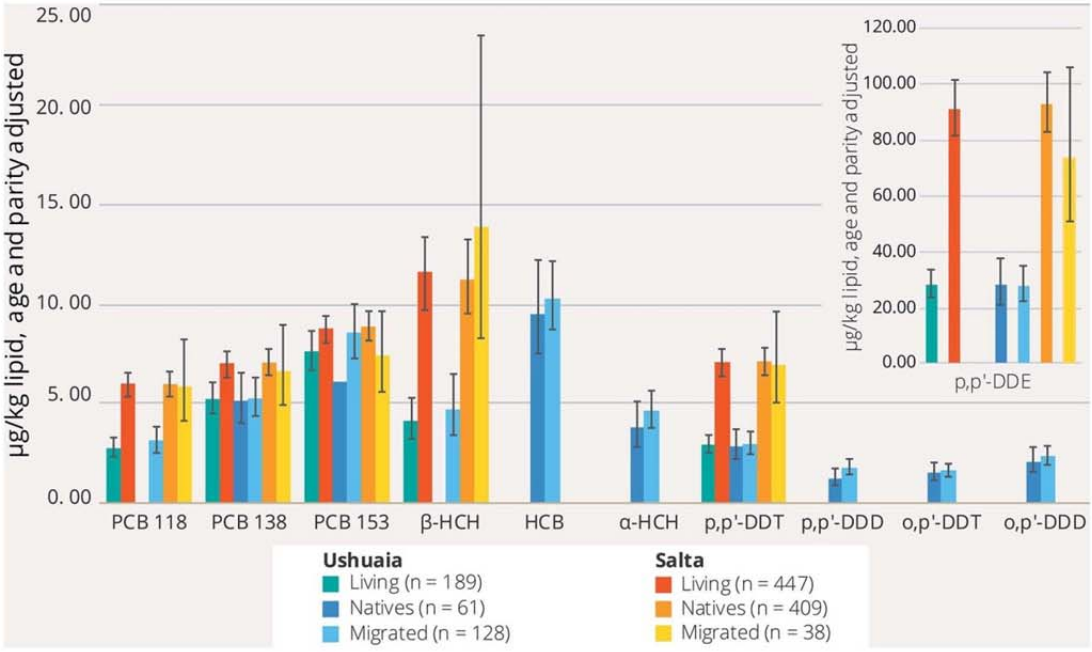

Fig. 1 Age- and parity-adjusted maternal serum OC concentrations (μg kg^{-1} lipid, geomean) with 95% CI error bars, stratified by residence (Ushuaia and Salta) and maternal birthplace (native or migrated) for compounds with a detection frequency > 60%. City differences (Ushuaia *versus* Salta) at $p < 0.001$ except for PCB 153 ($p = 0.081$). The p-values for differences between native and migrated groups are provided in Table 2; for full names of the compounds, see Section 2.2.

in Salta (a public institution responsible for all in-hospital deliveries in the city and the region).

The study (#2010/7317) was approved by the Ethics Committee of the Salta Medical Association and the Ministries of Health in both provinces. As required by law, the Norwegian Regional Committee for Medical and Health Research Ethics (REC North) approved the study (#2011/706), and it was conducted in accordance with the Helsinki declaration. Informed consent was obtained for any experimentation with human subjects in the study.

2.2 Chemical analysis

Blood collected in BD Vacutainers® (BD SST II Plus Advance 10/8.5 ml) was sampled and centrifuged at 2000 relative centrifugal force (RCF) for 10 minutes. The maternal serum was then transferred into vials (Sarstedt CryoPure, 2.0 ml tubes) for general analyses, or into glass vials (4.0 ml, pre-rinsed with *n*-hexane/acetone) retained for chemical analyses of the POPs. The serum samples were transferred in a frozen state to the EMASAR Biobank at the Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway.

The analytical work was conducted at the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain. Serum samples were analysed for the following organochlorines (OCs): pentachlorobenzene (PeCB); α- and β-HCB (hexachlorobenzene); α-, β- δ- and γ-HCH (hexachlorocyclohexane); 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o*,*p*′-DDT); 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p*,*p*′-DDT); 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o*,*p*′-DDD); 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p*,*p*′-DDD); 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene (*o*,*p*′-DDE); 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p*,*p*′-DDE); and polychlorinated biphenyl (PCB) congeners 28, 52, 101, 118, 138, 153 and 180. The analytical procedures employed have been described elsewhere by Bravo *et al.* (2017).[14] Briefly, recovery standards TBB and PCB-209, *n*-hexane and concentrated H_2SO_4 were added to the serum samples (1 ml). After vortexing and centrifugation, repeated extraction of the serum *n*-hexane layer followed and the combined extracts were reduced to near dryness. The remaining solution was quantitatively transferred to gas chromatograph (GC) vials using four 25 μl rinses of isooctane and the injection standard PCB-142 (20 μl) was added. Instrumental analysis was performed by gas chromatography with electron capture detection. Compound identification was confirmed by analysis with a GC coupled to a mass spectrometer. A blank was analysed for every 6 samples. The recoveries of the surrogate standards were 76 ± 13% and 82 ± 14% for PCB-30 and PCB-200, respectively. The measured concentrations of the compounds were adjusted for the recoveries of these standards. The limits of detection were calculated as three times the standard deviation of the noise measured next to the chromatographic peaks of each analyte: they ranged from 0.01 to 0.027 μg l^{-1} (Table 1). Moreover, the analytical methods fared well (within 20% variability of the consensus values) in the AMAP Ring Test Proficiency Program for Persistent Organic Pollutants in human serum conducted by the Centre de Toxicologie Institut National de Santé Publique du Québec.[15]

Chemical analyses of the serum lipid profile (Økland *et al.*, 2017)[13] were done enzymatically at the respective hospitals.

Table 1 Concentrations (in μg kg^{-1} lipid) and limits of detection of OCs in serum samples of delivering mothers from Argentina (2011–2012)

		Ushuaia (n = 199)						Salta (n = 471)						
Compound[a]	LOD[a]	% ≥ LOD	GM	AM	Median	Min	Max	% ≥ LOD	GM	AM	Median	Min	Max	*p*-Value[b]
PCB28	0.010	32.7	1.3	2.1	0.9	0.3	14.2	24.0	1.2	1.7	0.9	0.4	17.4	
PCB52	0.005	38.2	1.04	2.84	0.47	0.21	37.9	33.1	0.94	2.68	0.50	0.22	59.3	
PCB101	0.001	56.3	0.70	2.59	0.72	0.06	25.2	16.1	0.18	0.46	0.13	0.06	16.1	
PCB118	0.011	64.8	3.1	5.6	3.8	0.5	66.9	79.0	5.7	9.1	7.1	0.6	163	<0.001
PCB138	0.002	97.0	6.32	8.84	7.06	0.13	45.4	97.0	6.36	9.91	7.04	0.18	172	0.900
PCB153	0.008	97.0	9.13	12.19	9.80	0.48	51.7	94.3	8.08	11.60	8.80	0.48	144	0.091
PCB180	0.011	36.7	1.7	3.2	1.0	0.4	38.2	30.1	1.7	3.4	1.1	0.5	62.5	
p,*p*′-DDT	0.005	89.4	3.22	14.29	3.47	0.31	1950	97.0	6.83	12.32	6.32	0.31	334	<0.001
p,*p*′-DDE	0.013	99.0	38.5	196.5	32.7	1.2	23 800	100	80.2	228.3	69.0	3.69	12 100	<0.001
p,*p*′-DDD	0.002	81.4	1.63	2.89	2.41	0.13	71.5	52.0	0.60	1.17	0.67	0.09	23.3	
o,*p*′-DDT	0.005	66.3	1.61	2.71	2.22	0.26	15.3	15.7	0.55	0.94	0.43	0.20	30.5	
o,*p*′-DDE	0.013	47.7	2.2	3.4	1.4	0.5	22.5	15.3	1.4	2.2	1.2	0.6	57.5	
o,*p*′-DDD	0.007	68.8	2.31	4.02	2.97	0.32	29.9	24.2	0.95	1.56	0.66	0.30	21.3	
∑DDT			60.0	223.8	49.9	8.1	25 900		99.5	246.5	83.2	9.7	12 400	<0.001
PeCB	0.006	22.6	0.72	1.18	0.52	0.20	11.8	4.20	—[c]	—[c]	0.51	0.26	9.7	
HCB	0.027	80.9	10.0	19.0	9.8	1.4	499	57.1	5.9	8.9	7.1	1.5	102	
α-HCH	0.007	86.9	4.42	7.64	4.84	0.37	59.9	33.5	1.44	4.77	0.67	0.32	47.2	
β-HCH	0.010	61.3	6.0	21.2	7.8	0.5	281	71.3	9.4	31.0	13.7	0.5	483	0.003
δ-HCH	0.020	17.6	2.1	3.7	1.7	0.7	45.7	19.5	2.7	6.0	1.8	0.9	74.4	
γ-HCH	0.013	19.1	—[c]	—[c]	1.1	0.4	24.7	1.1	—[c]	—[c]	1.1	0.6	26.6	

[a] For the full names of the compounds see Section 2.2; the limit of detection (LOD) is in μg l^{-1}. [b] Mann Whitney test for compounds with a detection frequency > 60%. [c] Detection frequency < 20%.

Table 2 Fractional change in serum concentrations (in μg kg^{-1} lipid) of OCs by place of maternal birth, adjusted for age and parity[a,b,c]

		PCB 153					PCB 138					PCB 118				
Groups	*n*	GM	Ratio	*p*-Value	CI 95%		GM	Ratio	*p*-Value	CI 95%		GM	Ratio	*p*-Value	CI 95%	
Ushuaia natives	61	6.07	0.68	**0.008**	0.52	0.91	5.14	0.73	**0.010**	0.57	0.93					
Ushuaia migrated	128	8.55	0.96	0.679	0.81	1.14	5.25	0.75	**0.008**	0.61	0.91	3.13	0.53	**<0.001**	0.42	0.67
Salta natives	409	8.87	Ref.				7.01	Ref.				5.93	Ref.			
Salta migrated	38	7.36	0.83	0.200	0.61	1.13	6.62	0.94	0.625	0.77	1.19	5.83	0.98	0.929	0.68	1.40
Age, year			1.05	**<0.001**	1.03	1.06		1.06	**<0.001**	1.04	1.07		1.02	**0.013**	1.01	1.04
Parity			0.82	**<0.001**	0.76	0.87		0.78	**<0.001**	0.72	0.84		0.88	**0.001**	0.81	0.95

		p,*p*′-DDT					*p*,*p*′-DDE					β-HCH				
Groups	*n*	GM	Ratio	*p*-Value	CI 95%		GM	Ratio	*p*-Value	CI 95%		GM	Ratio	*p*-Value	CI 95%	
Ushuaia natives	61	2.88	0.41	**<0.001**	0.30	0.53	28.4	0.31	**<0.001**	0.22	0.43					
Ushuaia migrated	128	3.00	0.42	**<0.001**	0.35	0.51	28.2	0.30	**<0.001**	0.24	0.38	4.7	0.42	**<0.001**	0.28	0.62
Salta natives	409	7.06	Ref.				92.9	Ref.				11.2	Ref.			
Salta migrated	38	6.93	0.98	0.864	0.78	1.24	73.5	0.79	0.206	0.56	1.14	13.9	1.23	0.424	0.71	2.11
Age, year			1.03	**0.001**	1.01	1.04		1.08	**<0.001**	1.06	1.10		1.09	**<0.001**	1.06	1.12
Parity			0.92	**0.012**	0.86	0.97		0.65	**<0.001**	0.60	0.70		0.56	**<0.001**	0.50	0.64

[a] Univariate analyses of variance models based on detection frequencies above 60% in each population group and bootstrap with *p*-values and 95% CI based on 2000 samples. [b] Blanks indicate detection frequencies below 60%, and the corresponding data were excluded from the analyses. [c] Bonferroni *post hoc* pairwise comparisons: for PCB 153, the Ushuaia natives/Ushuaia migrated concentration ratio was 0.71, $p = 0.022$; for PCB 118, the Ushuaia migrated/Salta migrated ratio was 0.54, $p = 0.004$; and for both *p*,*p*′-DDT and *p*,*p*′-DDE, the Ushuaia natives and Ushuaia migrated/Salta migrated ratios were ~0.40, $p < 0.001$.

Total lipid contents were calculated from the cholesterol and triglyceride concentrations: total lipids = 90 + 1.3 (cholesterol + triglyceride) mg dl^{-1}.[16]

2.3 Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics for Windows statistical package version 24 (SPSS Inc. Chicago, IL, USA). Descriptive details are reported as means, standard deviations, median and range or percentage, and relationships between variables were explored using Spearman's rank correlation analysis. Concentrations below the LOD were replaced by LOD/2. Our choice to limit the between-group statistical analyses to compounds with a prevalence > 60% is recommended by the US CDC.[17] Despite log 10 transformation, the OC distributions remained skewed positively to the right and were not normally distributed according to the Kolmogorov–Smirnov test and Q–Q residual plots. We therefore employed non-parametric statistics (the Mann–Whitney *U* test) for comparisons of OC concentrations between the two study sites. For related compounds, Levene's test demonstrated equal variances between the two study sites. In a univariate general linear model (UGML) adjusted for the continuous variables age and parity (excluding stillbirths), acceptable case-wise diagnostics were possible even though abruptions of normality were

Table 3 Spearman's rho (ρ) for interrelationships between serum concentrations of OCs by city of residence ($n = 636$)[a]

		PCB 118	PCB 138	PCB 153	*p*,*p*′-DDT	*p*,*p*′-DDE	*p*,*p*′-DDD	*o*,*p*′-DDD	*o*,*p*′-DDT	HCB	α-HCH
Ushuaia ($n = 199$)	PCB 138	**0.632****									
	PCB 153	**0.595****	**0.758****								
	p,*p*′-DDT	**0.222****	**0.339****	**0.301****							
	p,*p*′-DDE	**0.259****	**0.510****	**0.465****	**0.381****						
	p,*p*′-DDD	0.073	−0.106	0.118	**0.172***	−0.046					
	o,*p*′-DDD	**0.417****	**0.180***	**0.148***	0.046	0.084	**0.177***				
	o,*p*′-DDT	0.103	−0.008	0.142	**0.197****	0.021	**0.646****	0.119			
	HCB	**0.192****	**0.348****	**0.233****	**0.249****	**0.464****	−0.002	−0.070	0.011		
	α-HCH	0.099	−0.060	0.114	−0.115	−0.064	**0.534****	**0.305****	**0.401****	−0.046	
	β-HCH	**0.292****	**0.388****	**0.445****	**0.151***	**0.474****	−0.073	0.029	0.050	**0.321****	−0.068
Salta ($n = 471$)	PCB 138	**0.589****									
	PCB 153	**0.592****	**0.899****								
	p,*p*′-DDT	**0.223****	**0.420****	**0.343****							
	p,*p*′-DDE	**0.124****	**0.414****	**0.384****	**0.564****						
	β-HCH	**0.288****	**0.411****	**0.379****	**0.312****	**0.553****	**0.205****				

[a] Results are presented for compounds with a detection frequency > 60%. ** Significant correlation at the 0.01 or * 0.05 level (2-tailed).

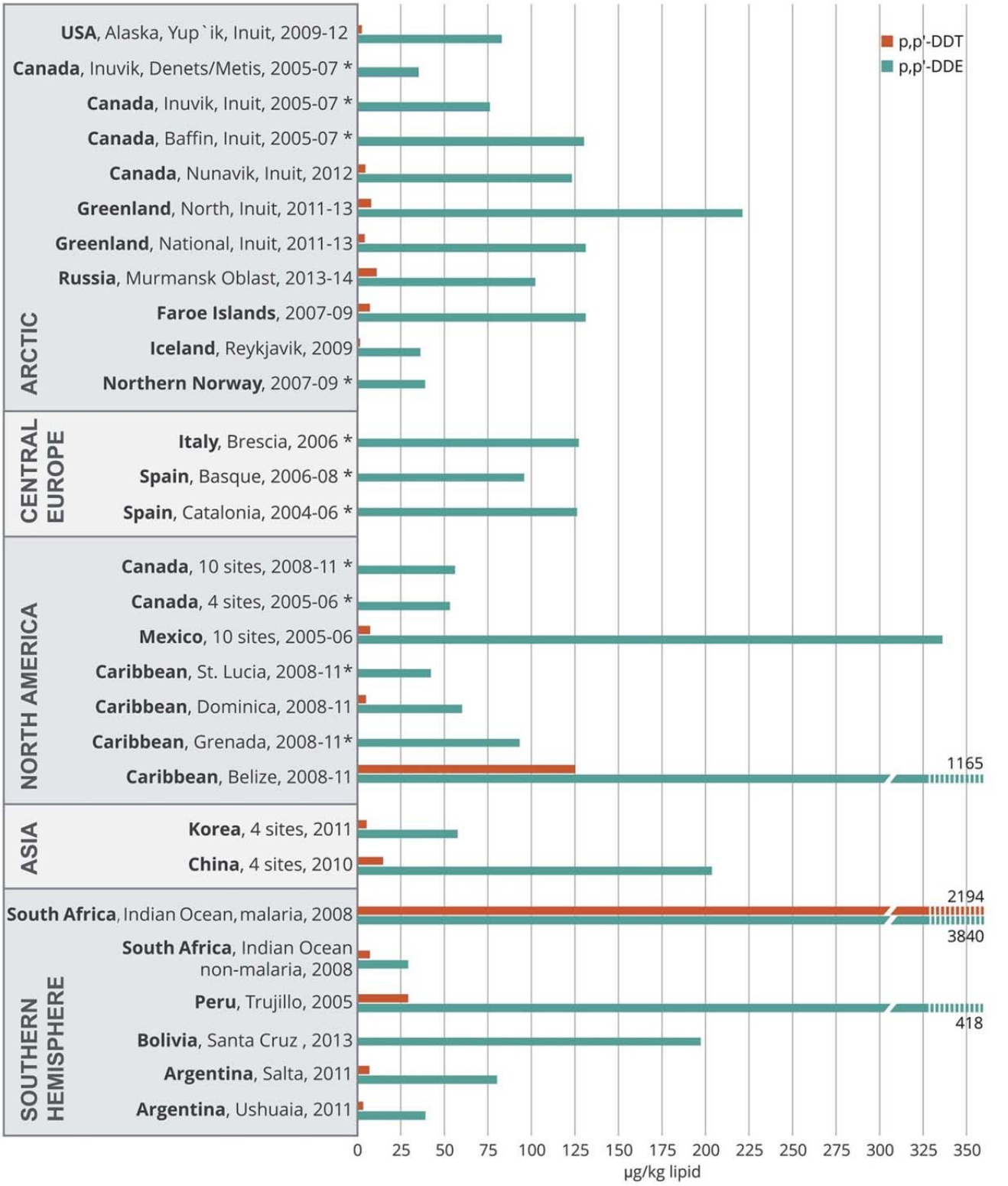

Fig. 2 Worldwide comparisons of serum or plasma *p,p'*-DDT and *p,p'*-DDE (µg kg^{-1} lipid, geomean) of pregnant or delivering women for the 2004–2014 period. **p,p'*-DDT, not reported due to high numbers of non-detections. For details and references, see the text and ESI S6 a and b.†

evident based on histograms, the Kolomogorov–Smirnov test and residual plots. Parity (excluding stillbirths) and months of lactation were highly correlated (Spearman's $\rho = 0.937$), with the former displaying the highest correlation with OC concentrations; it was thus selected in the modelling. Nevertheless, lactation yielded similar results (not reported). To harmonize

the group size, and thus the distribution, bootstrapping with 2000 estimates was selected. Between city comparisons (Ushuaia *versus* Salta) and maternal birthplaces involved four groups: (i) born and living in Ushuaia (natives, $n = 61$); (ii) living in Ushuaia, but born in another province within Argentina (migrated, $n = 128$); (iii) living and born in Salta (natives, $n = 409$); and (iv), living in Salta but born in another province within Argentina ($n = 38$). In the regression model, the coefficients for the OCs were estimates of the mean difference in log-transformed concentrations between each group (city or heritage) and the reference level. The regression coefficients were back-transformed (10^{β}) to reflect the ratios of change in concentrations, and in the text are described as % change = $(10^{\beta} - 1) \times 100$. The significant levels were set at $p < 0.05$. Finally, the ratios within the DDT group were calculated to distinguish ongoing chronic exposure *versus* dietary exposure or distant past exposure.[3,18]

3. Results

3.1 Personal characteristics

The personal characteristics of all participating women ($n = 698$) are described elsewhere[13] and include details about mothers (*e.g.*, age, marital status, education, employment, smoking habits, diet, and vitamin intake) and newborns. As mentioned, 28 participants were excluded from the current study cohort. With reference to Økland *et al.* (2017),[13] Salta women were younger, had somewhat higher parity, breastfed longer, had less education, and differed somewhat in dietary habits. Details about migration from other regions of Argentina

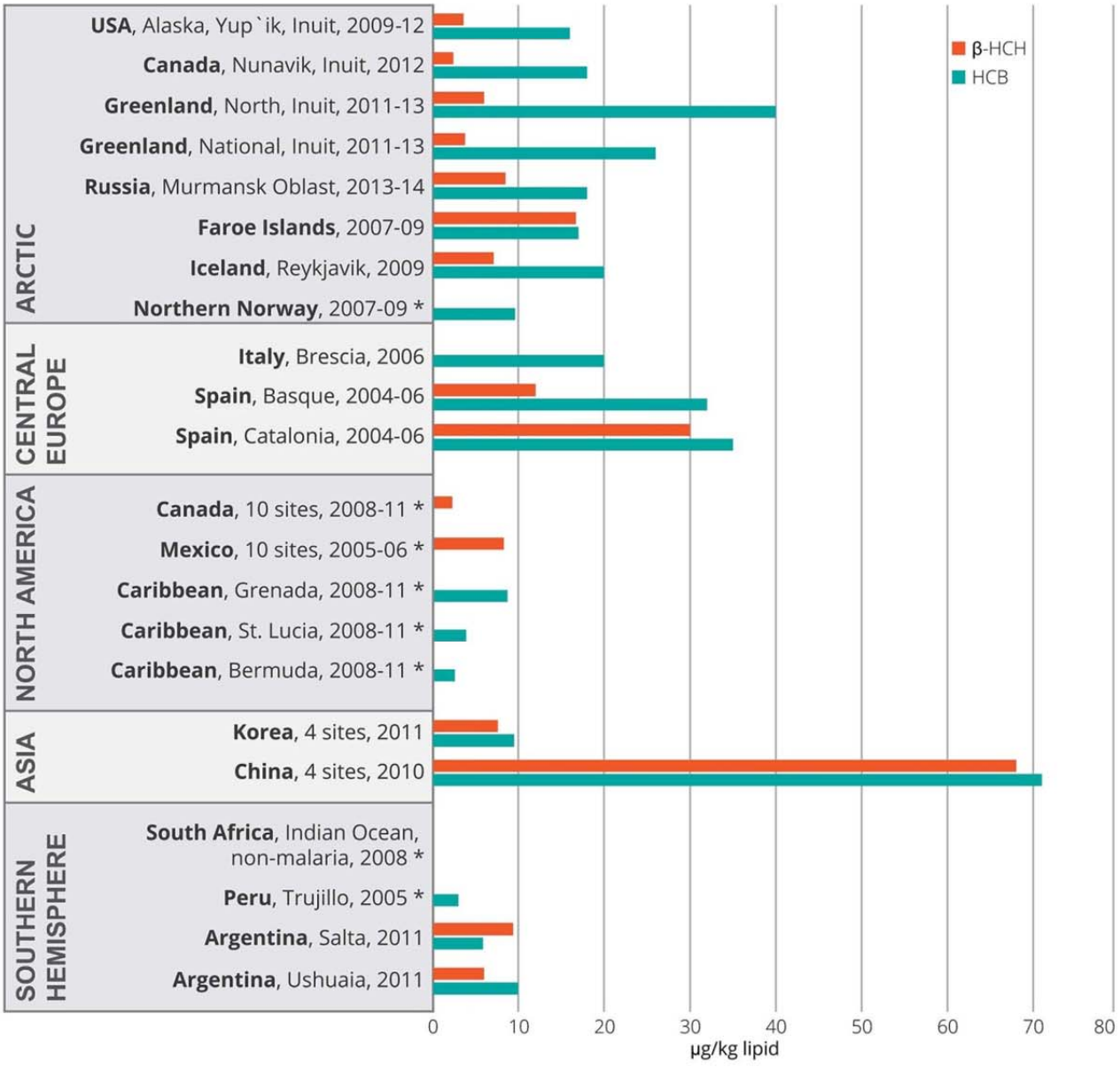

Fig. 3 Worldwide comparisons of serum or plasma β-HCH and HCB (µg kg^{-1} lipid, geomean) of pregnant or delivering women for the 2004–2014 period. *Not reported due to high numbers of non-detections. For details and references, see the text and ESI S6c and d.†

into the provinces of Ushuaia and Salta were also available for 189 and 446 participants, respectively. Almost everyone in Salta (91%) was born in the province compared to 32% in Ushuaia. Details about age, parity, breastfeeding and years of living in current home are reported in ESI Table S1.†

3.2 Influence of area residence and maternal birth place in OC serum concentrations

3.2.1 Detection frequency of OCs. The detection limits and frequencies are reported in Table 1. Among the 19 OCs analyzed, only six compounds had a detection rate above 60% in both places, specifically PCB 118, PCB 138 and PCB 153, *p*,*p*′-DDT, *p*,*p*′-DDE and β-HCH. Five additional compounds exceeded this threshold in Ushuaia, namely *p*,*p*′-DDD, *o*,*p*′-DDT, *o*,*p*′-DDD, HCB, and α-HCH. We selected *p*,*p*′-DDE, PCB 138, PCB 153 and *p*,*p*′-DDT which exhibited detection frequencies (>89%) for detailed evaluations, and a somewhat more inclusive group when considering maternal birthplaces (Table S2†).

3.2.2 Serum lipid-adjusted OC concentrations by city of residence. Of the OCs, lipid-adjusted serum *p*,*p*′-DDE (μg kg^{-1}

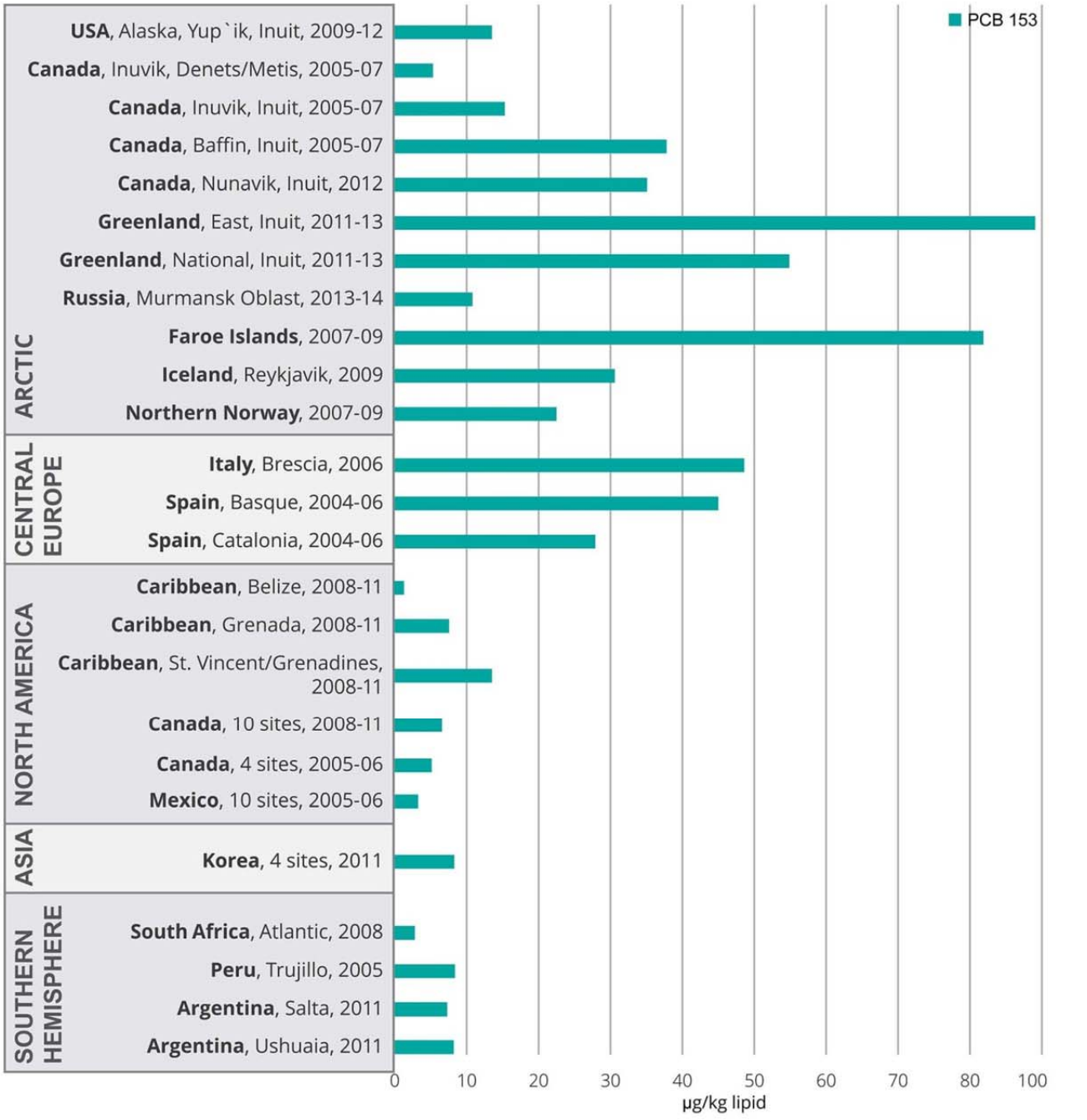

Fig. 4 Worldwide comparisons of serum or plasma PCB 153 (μg kg^{-1} lipid, geomean) of pregnant or delivering women for the 2004–2014 period. For details and references, see the text and ESI S6e.†

lipid) was the dominant compound at both sites (Table 1) followed by PCB 153 (respectively, lower by factors of 4.3 in Ushuaia and 9.9 in Salta based on GMs). Compared to Ushuaia, serum concentrations of PCB 118, β-HCH, *p*,*p*′-DDT, *p*,*p*′-DDE and ∑DDT were all higher ($p < 0.003$) in Salta, while there were no city differences for PCB 153 and 138. Wet-weight serum OC concentrations are summarized in Table S3.†

3.2.3 Impact of residence and place of birth on age- and parity-adjusted OC concentrations. The age- and parity-adjusted concentrations of the dominant OCs (ng per lipids) by area of residence are shown in Fig. 1 and are further stratified whether born in the two study areas or migration into them from other regions of Argentina ($n = 636$). Through bootstrapping, differences in OC concentrations between the Ushuaia and Salta women were shown to be distinct ($p < 0.001$), with the exception of PCB 153 ($p = 0.081$).

Several significant statistical differences ($p \leq 0.010$) were observed when considering residence history in terms of being native born or migrants to the two regions of Argentina (Table 2). Relative to Salta natives, those of Ushuaia had 27% lower concentrations of PCB 138 and 32% of PCB 153; and migrants into Ushuaia also exhibited reduced concentrations of PCB 118 and PCB 138 (respectively, by 47% and 25%), but not so for PCB 153. In addition, Ushuaian migrants had 46% less serum PCB 118 than those in Salta. The *p*,*p*′-DDE and *p*,*p*′-DDT concentrations for Ushuaian natives and migrants were less substantive by 60% and 70% when compared to the respective groups in Salta (Table 3). Ushuaia migrants had 58% lower concentrations of β-HCH compared to Saltanean natives (*post hoc* analysis). Intra-city differences in OC concentrations between native and migrated women in Salta did not reach statistical significance; nor for Ushuaians, with the exception that PCB concentrations in native citizens were on average 29% lower (Table 2; also see Table S4†). Age had a positive influence on OC concentrations and parity a negative one ($p < 0.05$). The statistical significance for these variables was lost for Ushuaia residents when examining each community separately (*e.g.*, Table S5†), but persisted for all OCs in Salta (data not shown).

3.3 Ratios of DDT-isomers

The *p*,*p*′-DDE/*p*,*p*′-DDT ratio was rather similar in Ushuaia (mean 22.8, median 11.4) and Salta (mean 19.2, median 11.6) women ($p > 0.05$, Table S4†). In both places, around 18% of the women had a ratio below 5.0. By comparison, the respective median ratios (0.09) of *p*,*p*′-DDT/*p*,*p*′-DDE were identical ($p > 0.05$); three women in Ushuaia and five in Salta exceeded a ratio of 1.00. In addition, the mean *o*,*p*′-DDT/*p*,*p*′-DDT ratio was calculated only for samples with concentrations above the LOD ($n = 183$) and had values of 1.09 (Ushuaia) and 0.29 (Salta) with $p < 0.001$.

3.4 Correlations of OC concentrations

Based on Spearman's rho tests, significant inter-OC correlations might be designated as weak ($\rho < 0.4$) to moderate (ρ 0.4–0.9) for the 5 OCs compared in Salta (Table 3). The most robust relationships occurred between PCB congeners ($\rho = 0.59$–0.90). For Ushuaia, the intra-PCB relationships were again the most robust ($\rho = 0.60$–0.76); others were mostly weak or absent.

3.5 Global comparisons

Our OC concentration data are summarized and compared to those reported for other regions of the world in Fig. 2–4; the corresponding world-wide data are compiled in Tables S6a–e and in Tables S6f & g† for PCB 138 and 118. This overview roughly covers a 10 year period (2004–2014). It is clear that the observed concentrations for the Ushuaia and Salta study participants are in the middle to lower range.

4. Discussion

4.1 Preamble

In addition to comparing our serum OC concentrations with those reported for other regions of the world, the influences of the following predictor variables are discussed below: maternal age, parity, breastfeeding history, province lived in and of birth (specifically, within the provinces of Ushuaia and Salta or migration into them from other Argentinian provinces), as well as dietary and environmental predictor variables.

4.2 The signature of the OCs in a global and regional comparison

4.2.1 DDT-group. In general, the observed maternal lipid-adjusted serum concentrations of *p*,*p*′-DDE in Salta may be designated as moderate when compared with those reported for other world areas (Fig. 2). By comparison, concentrations for Ushuaian mothers were in the lower range and comparable to those reported for Arctic populations not consuming marine mammals,[3] although somewhat higher than the lowest observed such as for the residents of the coast of non-malaria South Africa.[19] The concentrations for Saltanean mothers were comparable to those reported for Alaska Yup'ik and for Baffin Island and Canadian Inuits living in Inuvik,[3] although higher than in Korea[20] and central Canadian cities[21] – and both above and below those reported for the Caribbean islands.[22] The Argentinian maternal levels were moderately lower than in Western Australia (wet weight, wwt),[23] central Europe like Spain[24] and Italy,[25] and considerably lower than in Bolivia,[26] China,[27] and Arctic women with a traditional diet of marine mammals.[3] By contrast, the observed concentrations were considerably lower than in countries with historical use of DDT and subsequent prohibition such as Mexico[28] and Peru,[17] Vietnam (wwt)[29] and Belize.[22] Malaria-endemic areas of South Africa with active DDT treatments have reported the highest concentrations in contrast to the low levels in the neighboring non-malaria Indian Ocean region.[19]

The Argentinian *p*,*p*′-DDT concentrations observed (Fig. 2) were lower than in countries with a previous or current history of DDT usage.[17,19,22,27,29] Comparable *p*,*p*′-DDT levels to those in Salta have been observed in Mexico,[28] Northern Greenland and Faroe Island[3] and the non-malaria Indian Ocean area in South Africa.[19] The Ushuaian levels were slightly higher than those found in Alaska and Iceland.[3] Finally, our observed

concentrations of DDT-related compounds were higher (*p*,*p*′-DDE), comparable (*p*,*p*′-DDT) or lower (*p*,*p*′-DDD) than those reported for an adult population of Buenos Aires in 2006.[12]

Relative to Ushuaia, the higher concentrations of *p*,*p*′-DDT (and thus also *p*,*p*′-DDE) in Salta are in agreement with the nearly ten-fold higher use of insecticides in the home and perhaps in agriculture.[13] Several decades ago this compound was used for malaria vector control in the northwestern regions.[4] The current presence of low levels *p*,*p*′-DDT and the relatively high concentrations of its derivative *p*,*p*′-DDE point to past comprehensive use and ongoing entry into the local food web.[4,6,7,9] The observed *p*,*p*′-DDE/*p*,*p*′-DDT ratio of around 20 in the two communities (Table S4†) is consistent with historical use of the latter.[18] Nevertheless, chronic or more recent exposure to DDT cannot be excluded as one in five women in both communities had ratios below 5. The *o*,*p*′-DDT/*p*,*p*′-DDT ratio is a useful index in regions with active use of dicofol.[30] In Ushuaia, the relatively high *o*,*p*′-DDT/*p*,*p*′-DDT ratio of 1.09 compared to 0.29 in Salta (Table S4†) suggests some recent DDT input,[18] and is likely related to dicofol use.[30–32] Atmospheric release of dicofol in the province of Mendoza in western-central Argentina has also been reported,[9] and this insecticide still appears to be in use.[10] Furthermore, long-range transport of the relatively volatile Dicofol from neighboring regions and countries, or other southern continents, might also have some influence.[33,34]

4.2.2 HCB and the HCH-group. The observed concentrations of HCB and β-HCH (Table 1) were mostly in the low range compared to other jurisdictions (Fig. 3). In Ushuaia, the concentrations of both compounds were comparable to those for the women residents of Korea[20] and HCB concentrations were comparable to those reported in Northern Norway.[35] Serum HCB concentrations for mothers in Italy,[25] Spain[24] and remote Arctic regions[3] were up to two-fold higher, and up to six-fold in China.[27]

Before the use of pure lindane (γ-HCH) technical mixtures were used, which encompassed isomeric compositions of 60–70% α-HCH, 5–12% β-HCH and 10–15% γ-HCH.[36] Generally speaking, data on human maternal body burdens of α-HCH are scarce and are low or below the detection limit for mothers in China,[27] Australia,[23] Canada and Mexico.[28] Our Argentinian levels (Table 1), particularly those in Ushuaia, are comparable in magnitude to those reported in South Africa (2 μg kg^{-1} lipid)[37] and China (5.2 μg kg^{-1} lipid).[38] As depicted in Fig. 3, the observed β-HCH concentrations were higher than those from Canada, Greenland and Alaska,[3] comparable to those from Mexico,[28] Korea[20] and South Africa,[39] but lower than those reported for the Faroe Island[3] and Spain.[24] The high concentrations observed in China are consistent with its extensive organochlorine pesticide production and use.[27] The generally low β-HCH concentrations among Arctic populations contrast those of other OCs, and perhaps reflect decades of worldwide restrictions and prohibitions.

The observed HCB, α-HCH and β-HCH concentrations likely reflect regional diversities in past emissions. HCB is still released into the Argentinian environment as a by-product of pesticide use, various industrial activities, open burning processes, waste disposal and landfills.[9] The 3-fold higher α-HCH levels in Ushuaia, and its lack of a relationship with HCB, PCBs, *p*,*p*′-DDT and *p*,*p*′-DDE (Table 3) may reflect source-specific pathways. It is known that α-HCH is retained less in nature and humans than β-HCH.[36] Their comparable concentrations in Ushuaia mothers remain unexplained, as is the observation in China that α-HCH levels exceeded those of β-HCH.[38] Atmospheric long-range transportation of this semi-volatile HCH to colder climates may be relevant. Furthermore, the relatively higher exposure to the water-insoluble β-HCH in Salta suggests greater historical local usage of HCH pesticides compared with more central and southern remote areas. The positive correlations of β-HCH at both sites with both PCBs and *p*,*p*′-DDE (see Table 3) suggest common sources of exposure, while the scarcity of γ-HCH is consistent with its lower bioaccumulation potential and more rapid degradation in nature.[36] By contrast, a study in South Africa showed almost non-detectable maternal concentrations of HCB, α-HCH and β-HCH and a complete predominance of γ-HCH – this was explained by on-going use of lindane.[37]

4.2.3 PCB-congeners. PCB congeners 153 and 138 were the dominant PCBs, and this is like the footprint observed elsewhere. Furthermore, the observed PCB 118/PCB 180 ratio of 2–3 contrasts those observed elsewhere, such as in the Arctic (though with some Russian observations exempted),[3] but is in line with previous studies within Argentina.[6] Presumably this ratio reflects local use of technical mixtures with congener-specific composition.[6,11]

Despite prohibitions, Argentinian electric power-generation equipment, aged industrial plants, stockpiles and the other sources already mentioned continue to be sources of PCBs and continue to impact the environment and thus the food chain.[9,11] However from a global perspective, the Argentinian production and use of PCBs have been low. It is estimated that only 3% of the total global historical use of PCB occurred in the Southern Hemisphere, with Argentina contributing 0.1%.[40] This is consistent with the data for PCB 153 in Fig. 4.

4.3 Factors influencing OC concentrations

Generally speaking, we conclude that the exposures in Salta for the dominant OCs exceeded those in Ushuaia, with PCB 153 exempted. Taken together, the relatively robust inter-correlation patterns for PCBs, *p*,*p*′-DDE and β-HCH in both communities (Table 3) suggest more or less a common exposure pathway. However, in Ushuaia they are somewhat more limited for α-HCH, *p*,*p*′-DDD and *o*,*p*′-residues and suggest additional sources. The impact of inter-country migration was not strong, but nevertheless measurable. Ushuaian migrants had considerably lower serum PCB 118, *p*,*p*′-DDE and *p*,*p*′-DDT than those who had moved into Salta. Interestingly, PCB-153 appears to have contributed considerably to the overall population body burden of PCBs in Ushuaia. Geographic differences in OC levels as observed likely also reflect history, socioeconomic, culture, physiological factors, lifestyle and dietary differences.[22,24,41–43]

Age as a positive predictor of serum OC concentrations and parity and previous breastfeeding as negative ones are well

understood.[35,44] Because of the relatively long half-lives (in years) of OCs (typically > 7 years),[45] body burdens increase over time. OC storage in lipid tissues and loss *via* breastfeeding explain the observed negative impact of parity.

In Argentina, dietary foodstuffs are typically produced within the nation, but with regional differences. Extensive importation of foods into Ushuaia suggests an additional contrasting factor. OCs are often associated with animal lipid-rich aquatic and terrestrial food web sources, including dairy products and eggs, or even fruit, vegetables and grains.[35,41,46] As indicated by others[6] and confirmed in our recent publication,[13] fish contributed only 10–15% of the Argentinian diet. Argentinian studies have also suggested that freshwater species contain OCs due to the pollution of rivers.[6,11] Consumption of fatty meats and freshwater fish has been identified as a dietary predictor variable of OCs in Argentinian women.[47] Such sources would likely affect the inland residents of Salta more.

Salta province in the north has a subtropical climate, while Tierra del Fuego is at a lower latitude and has a cooler climate. Its prevailing winds are from the south-west, and thus a latitude effect equivalent to that operative in the Arctic region seems unlikely. However, Ushuaia's expanding industry, economy, population and tourism constitute environmental challenges that include heavily polluted waterfronts and limited waste-disposal systems.[8]

4.4 Strengths and limitations

The present study of OCs in two distinct provinces of Argentina uses the methodological and analytical approaches employed in AMAP studies and our laboratory participated successfully in the most recent AMAP Analytical Ring Test.[15] Nearly all invited women participated, and the sample sizes for the two regions are comparable to those for other population monitoring studies. We consider the representativeness of our sample set as acceptable. Our findings would facilitate the planning of other surveys of other regions of Argentina, as its generalizability might best be limited to the investigated regions.

Low detection frequencies for some compounds might have introduced some bias into the statistical analyses. A few cases of extreme values of DDTs were also found, but sensitivity analyses supported their inclusion. We acknowledge that our comparisons of our data with those of multiple studies are subject to uncertainties related to varying methodological and analytical variances. However, most of the publications referred to employed the AMAP study approach, and the respective laboratories have participated in the mentioned Inter-laboratory Ring test.[3] Our use of lipid-adjusted concentrations in our comparisons might have helped to minimize bias such as that associated with different pregnancy sampling periods.[18,44]

5. Conclusions

To the best of our knowledge, the EMASAR study is the first conducted in Argentina on OC concentrations in the sera of women related to pregnancy. Overall, our results indicate that the serum OC concentrations in Argentinian delivering women were in the lower range. Even so, the geographical distributions of the DDTs, HCB, HCHs and PCBs for the Argentinian southernmost and northwest mothers are inferred to reflect differences in domestic sources, regional diversity in historical and current uses, prior and current industrial emissions, and potential contributions of disposal sites, stockpiles and long-range transfer. Due to the wide range of pesticide use, some individuals are deemed to be at higher risk. Compared to other countries, the natives of Ushuaia in the Tierra del Fuego province are considered to be subject to lower levels of POPs than in many other countries. By comparison, the exposure of the Salta group is considered to be moderately elevated. The relatively high PCB 118/PCB 180 ratio observed for both Argentinian communities contrasts that seen elsewhere and likely reflects the use of technical mixtures with congener-specific composition.

Funding

We acknowledge The Norwegian Department of Foreign Affairs and the Arctic Monitoring and Assessment Programme (AMAP) (2011/706-13) for generously funding the study.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge the Health Ministries of the provinces Salta and Tierra del Fuego, Argentina for their administrative support and facilitation. We acknowledge Dr Martin de la Arena for his assistance in obtaining the ethics approval and initiating the study in Argentina, Bente A. Augdal for designing the biological sampling procedures and managing the study biobank at the UiT, Dra. Silvia dib Ashur for implementing the biological sampling routines, and Lic. Maria José Aleman (Salta) and Maria Florence Bressan (Ushuaia) for their key roles in the local administration of the study. Finally, we acknowledge the participants from Ushuaia and Salta.

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MATERIAL SUPLEMENTARI

Variations in serum concentrations of selected organochlorines among delivering women in Argentina. The EMASAR study.

Taula de continguts:

Table S1. Personal characteristic of the study population by city of living and province of maternal birth (n = 636)

	Ushuaia natives (n = 61)				Ushuaia migrated (n = 128)				Salta natives (n = 408)				Salta migrated (n = 38)				
	n	*Mean or n (SD or %)*	*Median*	*min-max*	*n*	*Mean or n (SD or %)*	*Median*	*min-max*	*n*	*Mean or n (SD or %)*	*Median*	*min-max*	n	*Mean or n (SD or %)*	*Median*	*min-max*	*p-value*
Age	61	25.2 (6.1)	24.6	16-38	128	30.2 (6.2)	29.6	16-45	409	24.3 (6.1)	23.1	14-44	38	24.3 (6.5)	24.4	15-40	<0.001
Parity	61	1.7 (0.81)	1	1-4	128	1.95 (1.02)	2	1-7	409	2.2 (1.5)	2	1-8	38	1.9 (1.1)	2	1-6	0.262
para 1		31 (50.8)				48 (37.5)				187 (45.7)				17 (44.7)			
para 2		21 (34.4)				51 (39.8)				97 (23.7)				14 (36.8)			
para ≥3		9 (14.8)				29 (22.7)				125 (30.6)				7 (18.4)			
Previous breastfeeding, months	30	20.1 (14.9)	18	0-60	80	18.7 (20.7)	14	0-156	215	34.9 (30.9)	24	0-217	21	26 (17.9)	24	2-77	<0.001
Year of living current home	61	10.8 (9.5)	10	1-32	128	4.8 (5.7)	3	1-28	408	11.9 (9.7)	10	1-42	38	6.3 (6.5)	3.5	1-22	<0.001

Table S2. Detection frequencies of OCs in serum of delivering Argentinean women, by residence and maternal provinces of birthplace (n = 636)

	Living Ushuaia			Living Salta		
	Total	**Ushuaia natives**	**Migrated**	**Total**	**Salta natives**	**Migrated**
Compound[a]	*% ≥ LOD*[a]	*% ≥ LOD*[a]	*% ≥ LOD*[a]	*% ≥ LOD*[a]	*% ≥ LOD*[a]	*% ≥ LOD*[a]
PCB 118	64.8	47.5	71.9	79.0	78.7	81.6
PCB 138	97.4	98.4	96.9	97.1	96.8	100
PCB 153	94.2	93.4	98.4	94.2	94.1	94.7
p,p'-DDT	89.4	83.6	92.2	96.9	96.6	100
p,p'-DDE	98.9	98.4	99.2	100	100	100
p,p'-DDD	81.0	73.8	84.4	51.2	51.6	47.4
o,p'-DDT	66.1	63.9	67.2	15.9	16.9	5.3
o,p'-DDD	68.8	63.9	71.1	24.9	25.5	18.4
HCB	81.5	77.0	83.6	58.4	58.4	57.9
α-HCH	86.2	83.6	87.5	33.9	32.5	36.8
β-HCH	60.8	54.1	64.1	71.7	71.6	84.2

[a]For the full names of the compounds, see Section 2.2 of the text. For the LODs, see Table 1 of the text.

Table S3. Concentrations (µg/L) of OCs in serum samples of delivering Argentinean mothers (2011-2012)

	Ushuaia (n = 199)					Salta (n = 471)					
Compound	**GM**[a]	**AM**[a]	**Median**	**Min**	**Max**	**GM**[a]	**AM**[a]	**Median**	**Min**	**Max**	**p-value**[b]
PCB28	0.01	0.01	<LOD	<LOD	0.09	0.01	0.01	<LOD	<LOD	0.10	
PCB52	0.007	0.019	<LOD	<LOD	0.206	0.006	0.015	<LOD	<LOD	0.322	
PCB101	0.005	0.016	0.005	<LOD	0.137	0.001	0.003	<LOD	<LOD	0.089	
PCB118	0.02	0.04	0.02	<LOD	0.37	0.03	0.05	0.04	0.01	0.89	<0.001
PCB138	0.041	0.057	0.045	<LOD	0.259	0.037	0.058	0.040	0.002	0.934	0.206
PCB153	0.059	0.079	0.064	<LOD	0.349	0.047	0.068	0.051	0.008	0.789	0.001
PCB180	0.01	0.02	<LOD	<LOD	0.24	0.01	0.02	<LOD	<LOD	0.34	
p,p'-DDT	0.021	0.081	0.022	<LOD	10.2	0.041	0.074	0.037	<LOD	2.62	<0.001
p,p'-DDE	0.25	1.10	0.22	0.01	124	0.47	1.30	0.42	0.03	78.9	<0.001
p,p'-DDD	0.011	0.019	0.015	<LOD	0.370	0.003	0.007	0.004	<LOD	0.180	
o,p'-DDT	0.010	0.018	0.014	<LOD	0.080	0.003	0.006	<LOD	<LOD	0.239	
o,p'-DDE	0.01	0.02	<LOD	<LOD	0.12	0.01	0.01	<LOD	<LOD	0.31	
o,p'-DDD	0.015	0.025	0.019	<LOD	0.146	0.006	0.009	<LOD	<LOD	0.124	
∑DDT	0.39	1.29	0.33	0.06	135	0.58	1.45	0.50	0.06	80.9	<0.001
PeCB	0.005	0.008	<LOD	<LOD	0.067	--[c]	--[c]	<LOD	<LOD	0.038	
HCB	0.07	0.12	0.07	<LOD	2.70	0.04	0.05	0.04	<LOD	0.69	
α-HCH	0.029	0.049	0.032	<LOD	0.317	0.008	0.027	<LOD	<LOD	0.286	
β-HCH	0.04	0.14	0.06	<LOD	1.40	0.06	0.18	0.08	<LOD	3.10	<0.001
δ-HCH	0.01	0.02	<LOD	<LOD	0.25	0.02	0.03	<LOD	<LOD	0.39	
γ-HCH	--[c]	--[c]	<LOD	<LOD	0.13	--[c]	--[c]	<LOD	<LOD	0.11	

[a]For the full names of the compounds, see Section 2.2 of the text; [b]Mann Whitney test for compounds with a detection > 60%; [c]Detection frequency < 20%. For LODs, see Table 1 in the text.

Table S4. Concentrations (μg/kg lipid) of OCs in serum samples of delivering Argentinian mothers by maternal province of birth (n = 636; 2011-2012)[a]

		PCB 118	PCB 138	PCB 153	*p,p'*-DDT	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDT	*o,p'*-DDD	HCB	*α*-HCH	*β*-HCH	Ushuaia (n = 189)[b]		
													DDE to DDT ratio	DDT to DDE ratio	o,p'-DDT to p,p'-DDT
Ushuaia natives (n = 61)	GM	2.3	5.50	6.41	2.94	32.2	1.24	1.53	2.13	8.0	3.68	3.6			
	AM	5.1	7.62	9.34	4.51	71.5	2.26	2.60	4.03	10.5	6.44	9.2			
	Median	1.2	5.71	6.53	3.55	24.8	1.88	2.16	2.59	8.8	3.41	3.6			
	Min	0.6	0.13	0.48	0.31	1.88	0.13	0.28	0.38	1.6	0.45	0.5			
	Max	66.9	37.2	40.2	16.3	516	10.5	11.3	26.2	30.4	33.9	59.0			
Ushuaia migrated (n = 128)	GM	3.5	6.91	10.7	3.39	41.7	1.83	1.64	2.36	11.2	4.70	7.5			
	AM	5.7	9.58	13.5	19.8	266	3.20	2.73	3.89	23.9	7.95	27.4	22.8	0.16	1.09
	Median	4.1	7.43	11.5	3.37	35.4	2.55	2.37	3.16	10.1	5.36	10.9	11.4	0.09	0.99
	Min	0.5	0.13	0.49	0.40	1.2	0.13	0.26	0.32	1.4	0.37	0.5	0.55	0	0.01
	Max	30.5	45.4	51.7	1950	23800	71.5	15.3	29.9	499	57.5	281	253	1.81	4.71
	p-value[c]		0.025	<0.001	0.856	0.085	0.055	0.784	0.493	0.09	0.143				
Salta natives (n = 409)	GM	5.7	6.35	8.18	6.78	80.3	0.60	0.57	0.98	6.1	1.41	9.6	Salta (n = 447)[b]		
	AM	9.2	10.1	11.8	12.7	240	1.21	0.99	1.63	9.2	4.66	31.8			
	Median	7.3	7.16	9.05	6.27	65.8	0.63	0.43	0.66	7.2	0.67	13.9	DDE to DDT ratio	DDT to DDE ratio	o,p'-DDT to p,p'-DDT
	Min	0.6	0.18	0.48	0.31	3.7	0.09	0.20	0.30	1.5	0.32	0.5			
	Max	163	172	144	334	12100	23.3	30.5	21.3	102	47.2	483			
Salta migrated (n = 38)	GM	6.0	6.86	7.57	7.02	78.2	0.57	0.44	0.82	5.9	1.61	15.4			
	AM	9.5	9.50	10.6	9.26	154	0.86	0.49	1.06	8.6	5.07	35.8	19.2	0.14	0.29
	Median	6.8	6.04	7.20	6.42	66.2	0.43	0.39	0.63	7.2	0.69	17.5	11.6	0.09	0.2
	Min	0.6	2.31	0.58	1.48	8.9	0.14	0.28	0.41	1.6	0.40	0.7	0.48	0	0.02
	Max	43.4	43.1	51.3	48.2	897	2.84	2.55	3.84	24.9	45.8	231	214	2.09	1.97
	p-value[c]	0.999	0.686	0.286	0.822	0.998						0.124			

[a]For the full names of the compounds, see Section 2.2 of the text; [b]For the o,p'-DDT to p,p'-DDT ratio, n = 114 in Ushuaia and n = 69 in Salta; [c]Mann Whitney test for compounds with a detection > 60%; natives versus migrated in each place.

Table S5. Fractional change in *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDD, HCB, and *α*-HCH serum concentrations (μg/kg lipid) per unit change, in place of maternal birth and adjusted for age and parity among delivering women from Ushuaia[a,b]

	p,p'-DDD						*o,p'*-DDT					*o,p'*-DDD				
	n	GM	Ratio	p-value	CI 95%		GM	Ratio	p-value	CI 95%		GM	Ratio	p-value	CI 95%	
Ushuaia natives	61	1.27	0.71	0.112	0.46	1.08	1.55	0.95	0.757	0.66	1.37	2.08	0.87	0.486	0.60	1.28
Ushuaia migrated	128	1.80	1.0		1.00	1.00	1.63	ref.				2.38	ref.			
Age, year			1.0	0.828	0.97	1.03		0.99	0.662	0.96	1.02		0.99	0.575	0.96	1.02
Parity			1.09	0.354	0.90	1.31		1.17	0.125	0.95	1.49		1.05	0.604	0.85	1.25

	HCB						*α*-HCH				
	n	GM	Ratio	p-value	CI 95%		GM	Ratio	p-value	CI 95%	
Ushuaia natives	61	9.53	0.92	0.552	0.69	1.24	3.79	0.82	0.285	0.57	1.19
Ushuaia migrated	128	10.3	ref.				4.62	ref.			1.0
Age, year			1.07	**<0.001**	1.04	1.10		1.01	0.657	0.98	1.04
Parity			0.78	**0.008**	0.65	0.90		1.05	0.547	0.87	1.23

[a]Univariate analyses of variance model based on detection frequencies above 60% in native and migrated groups, and bootstrapping with p-value and 95% CI based on 2000 samples; [b]For the full names of the compounds, see Section 2.2 of the text.

Tables S6 a-g Global comparisons of selected OCs for the 2004-2014 period

Table S6a. Worldwide comparisons of serum or plasma p,p'-DDE (µg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	p,p'-DDE	Unit	95% CI	Range	DDE:DDT ratio	Material	n	Period	Year	References
Argentina	Ushuaia	39	GM	33-46	0.5-23837	23	serum	199	2 days postpartum	2011	present study
	Salta	80	GM	72-90	4-12059	19	serum	471	2 days postpartum	2011-2012	present study
Bolivia	Santa Cruz de la Sierra	197	M			23	serum	200	delivery	2013	Arrebola et al., 2016
Peru	Trujillo	418	AM	255-686		19	serum	59	delivery	2004-2005	Adetona et al., 2013
South Africa	Indian Ocean, non-malaria	29	GM	25-33	8-343		plasma	117	delivery	2008	Channa et al., 2012
	Indian ocean, malaria	3840	GM	3008-4902	37-92559		plasma	91	delivery	2008	Channa et al., 2012
China	4 sites	204	M		<LOD-3194		serum	81	delivery	2010	Guo et al., 2014
Korea	4 sites	57	M				serum	105	delivery	2011	Kim et al., 2013
Caribbean	Belize	1165	GM	889-1526		9	serum	50	pregnancy	2008-2011	Forde et al., 2014
	Grenada	93	GM	71-122			serum	50	pregnancy	2008-2011	Forde et al., 2014
	Dominica	60	GM	46-80			serum	50	pregnancy	2008-2011	Forde et al., 2014
	St. Lucia	42	GM	35-55		13	serum	46	pregnancy	2008-2011	Forde et al., 2014
Mexico	10 sites	336	GM	295-382			plasma	240	3rd trimester	2005-2006	Adlard et al., 2014
Canada	4 sites	53	GM	48-58			plasma	103	1st trimester	2005-2006	Adlard et al., 2014
Canada	10 sites	56	GM	54-58	ND-5306		plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Spain	Catalonia, Sabadell	126	GM	118-135			serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
	Basque, Gipuzkoa	96	GM	90-102			serum	628	1st trimester	2006-2008	Ibarluzea et al., 2011
Italy	Brescia	127	GM	42-377			serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	39	GM		11-351		serum	508	2nd trimester	2007-09	Veyhe et al., 2015
Iceland	Reykjavik	36	GM		12-139	26	plasma	33	3rd trimester	2009	AMAP 2015
Faroe Islands	Faroe Islands	131	GM		6-1517	20	plasma	500		2007-2009	AMAP 2015
Russia	Murmansk	102	GM		16-1221		plasma	50		2013-2014	AMAP 2015
Greenland	National, Inuit	131	GM		16-1300	32	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
	North, Inuit	221	GM		18-990	29	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
Canada Arctic	Nunavik, Inuit	123	GM		11-520		plasma	112		2012	AMAP 2015
Canada Arctic	Baffin, Inuit	130	GM	110-150	17-670		plasma	100	3rd trim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Inuit	76	GM	59-98	ND-870		plasma	52	3rd trim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Denets/Metis	35	GM	26-49	13-140		plasma	17	3rd trim/delivery	2005-2007	Curren et al., 2015
USA, Alaska	Yup`ik, Inuit	83	GM		14-373		plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD, limit of detection; M, median; ND, non-detected

Table S6b. Worldwide comparisons of serum or plasma p,p'-DDT (μg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	p,p'-DDT	Unit	95% CI	Range	Material	n	Period	Year	References
Argentina	Ushuaia	3.2	GM	2.8-3.7	0.3-1952	serum	199	2 days postpartum	2011	present study
	Salta	6.8	GM	6.3-7.5	0.3-334	serum	471	2 days postpartum	2011-2012	present study
Peru	Trujillo	29	AM	18-45		serum	44	delivery	2004-2005	Adetona et al., 2013
South Africa	Indian Ocean, non-malaria	7	GM	6-7	4-37	plasma	117	delivery	2008	Channa et al., 2012
	Indian ocean, malaria	2194	GM	1706-2823	8-21856	plasma	91	delivery	2008	Channa et al., 2012
China	4 cities	14.7	M		<LOD-362	serum	81	delivery	2010	Guo et al., 2014
Korea	4 cities	5.2	M			serum	105	delivery	2011	Kim et al., 2013
Carribean	Belize	125	GM	87-179		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Dominica	4.8	GM	3.3-6.9		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Grenada, St. Lucia	NA	GM	NA		serum	50+50	pregnancy	2008-2011	Forde et al., 2014
Mexico	10 sites	7.1	GM	NA-8.4		plasma	240	3rd trimester	2005-2006	Adlard et al., 2014
Canada	4 sites	NA	GM			plasma	103	1st trimester	2005-2006	Adlard et al., 2014
Canada	10 sites	NA	GM			plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Spain	Catalonia/Basque	NA	GM			serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
Italy	Brescia	NA	GM			serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	NA	GM			serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Iceland	Reykjavik	1.4	GM		<1.3-5.7	plasma	33	3rd trimester	2009	AMAP 2015
Faroe Islands		7	GM		0.1-110	plasma	500		2007-2009	AMAP 2015
Russia	Murmansk Oblast	11	GM		1.3-376	plasma	50		2013-2014	AMAP 2015
Greenland	National	4.1	GM		2.0-68	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
	North, Inuit	7.7	GM		2.5-35	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
Canada Arctic	Nunavik, Inuit	4.4	GM		<LOD-33	plasma	112		2012	AMAP 2015
Canada Arctic	Baffin, Inuit	ND	GM	110-150	ND-18	plasma	22	3rd trim/delivery	2005-2007	Curren et al., 2014
	Inuvik, Inuit	ND	GM	ND	ND-11	plasma	18	3rd trim/delivery	2005-2007	Curren et al., 2014
	Inuvik, Denets/Metis	ND	GM	ND	ND	plasma	6	3rd trim/delivery	2005-2007	Curren et al., 2014
USA, Alaska	Yup`ik, Inuit	2.5	GM		<LOD-12	plasma	156		2007-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD, limit of detection; M, median; NA or ND, not reported due to high numbers of non-detected samples

Table S6c. Worldwide comparisons of serum or plasma HCB (μg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	HCB	Unit	95% CI	Range	Material	n	Period	Year	References
Argentina	Ushuaia	10	GM	8.7-11	1.4-499	serum	199	2 days postpartum	2011	present study
	Salta	5.9	GM	5.4-6.4	1.5-102	serum	471	2 days postpartum	2011-2012	present study
Peru	Trujillo	3	AM		2.4-3.8	serum	59	delivery	2005	Adetona et al., 2013
South Africa	Indian Ocean, non-malaria	NA	GM			plasma	117	delivery	2008	Channa et al., 2012
China	4 sites	71	M		<LOD-643	serum	81	delivery	2010	Guo et al., 2014
Korea	4 sites	9.5	M			serum	105	delivery	2011	Kim et al., 2013
Canada	10 sites	NA	GM			plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Caribbean	Bermuda	2.6	GM	2.3-3.0		serum	50	pregnancy	2008-2011	Forde et al., 2014
	St. Lucia	3.9	GM	3.4-4.6		serum	46	pregnancy	2008-2011	Forde et al., 2014
	Grenada	8.6	GM	7.4-10		serum	50	pregnancy	2008-2011	Forde et al., 2014
Spain	Catalonia, Sabadell	35	GM	33-38		serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
	Basque, Gipuzkoa	32	GM	30-34		serum	628	1st trimester	2006-2008	Ibarluzea et al., 2011
Italy	Brescia	20	GM	12-38		serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	9.6	GM		3.5-53	serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Iceland	Reykjavik	20	GM		12-35	plasma	33	3rd trimester	2009	AMAP 2015
Faroe Islands		17	GM		3-116	plasma	500		2007-2009	AMAP 2015
Russia	Murmansk Oblast	18	GM		5.3-252	plasma	50		2013-2014	AMAP 2015
Greenland	National, Inuit	26	GM		5.8-170	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
	North, Inuit	40	GM		5.8-130	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et.al., 2015
Canada Arctic	Nunavik, Inuit	18	GM		<LOD-110	plasma	112		2012	AMAP 2015
USA, Alaska	Yup`ik, Inuit	16	GM		2.7-99	plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD, limit of detection; M, median; NA, not reported due to high numbers of non-detected samples

Table S6d. Worldwide comparisons of serum or plasma β-HCH (µg/kg lipid) of pregnant or delivering women for the 2004-2013 period

Country	Region/area	β-HCH	Unit	95% CI	range	Material	n	Period	Year	References
Argentina	Ushuaia	6	GM	4.7-7.6	0.5-281	serum	199	2 days postpartum	2011	present study
	Salta	9.4	GM	8.0-11	0.5-483	serum	471	2 days postpartum	2011-12	present study
South Africa	Indian Ocean, non-malaria	NA	GM				117		2008	Channa et al., 2012
China	4 sites	68	M		<LOD-348	serum	81	delivery	2010	Guo et al., 2014
Korea	4 sites	7.6	M			serum	105	delivery	2011	Kim et al., 2013
Mexico	10 sites	8.3	GM	7.3-9.5		plasma	240	3rd trimester	2005-2006	Adlard et al., 2014
Canada	4 sites	2.1	GM	1.9-2.4		plasma	103	1st trimester	2005-2006	Adlard et al., 2014
Canada	10 sites	2.3	GM	2.2-2.4	ND-1108	plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Spain	Catalonia, Sabadell	30	GM	29-32		serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
	Basque, Gipuzkoa	12	GM	11-13		serum	628	1st trimester	2006-2008	Ibarluzea et al., 2011
Norway	Arctic	NA	GM			serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Faroe Islands		16.7	GM		2.0-110	plasma	500		2007-2009	AMAP 2015
Island	Reykjavik	7.1	GM		3.0-28	plasma	33	3rd trimester	2009	AMAP 2015
Russia	Murmansk Oblast	8.5	GM		0.8-146	plasma	50		2007-2009	AMAP 2015
Greenland	National, Inuit	3.8	GM		0.5-34	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
Greenland	North, Inuit	6	GM		0.5-34	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
Canada Arctic	Nunavik- Inuit	2.4	GM		<LOD-16	plasma	112		2012	AMAP 2015
USA, Alaska	Yup`ik - Inuit	3.6	GM		<LOD-37	plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean, GM, geometric mean; LOD, limit of detection; M, median; NA or ND, not reported due to high numbers of non-detected samples

Table S6e. Worldwide comparisons of serum or plasma PCB 153 (µg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	PCB 153		95% CI	range	Material	n	Period	Year	References
Argentina	Ushuaia	9.1	GM	1.1-10	0.5-52	serum	199	2 days postpartum	2011	present study
	Salta	8.1	GM	7.4-8.9	0.5-144	serum	471	2 days postpartum	2011-2012	present study
Peru	Trujillo	9.3	AM	7.2-12		serum	59	delivery	2004-2005	Adetona et al., 2013
South Africa	Atlantic	3.2	GM	2.7-3.8	0.7-21	plasma	61		2008	Rollin et al., 2009
Korea (median)	4 sites	9.2	M			serum	105	delivery	2011	Kim et al., 2013
Mexico	10 sites	3.6	GM	3.3-4		plasma	240	3rd trimester	2005-2006	Adlard, 2014
Canada	4 sites	5.7	GM	5.1-6.3		plasma	103	1st trimester	2005-2006	Adlard, 2014
Canada	10 sites	7.3	GM		ND-26	plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Caribbean	St. Vincent/Grenadines	15	GM	13-18		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Grenada	8.4	GM	7.1-9.9		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Belize	1.5	GM	1.3-1.8		serum	50	pregnancy	2008-2011	Forde et al., 2014
Spain	Catalonia, Sabadell	31	GM	29-32		serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
	Basque, Gipuzkoa	50	GM	48-53		serum	628	1st trimester	2006-2008	Ibarluzea et al., 2011
Italy	Brescia	54	GM	21-216		serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	25	GM		5.3-201	serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Faroe Islands		91	GM		1-694	plasma	500		2007-2009	AMAP 2015
Island	Reykjavik	34	GM		18-108	plasma	33	3rd trimester	2009	AMAP 2015
Russia	Murmansk Oblast	12	GM		1.3-57	plasma	50		2013-2014	AMAP 2015
Greenland	National, Inuit	61	GM		8.9-950	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
	East, Inuit	99	GM		8.9-950	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
Canada Arctic	Nunavik, Inuit	39	GM		2.4-230	plasma	112		2012	AMAP 2015
Canada Arctic	Baffin, Inuit	42	GM	36-50	6.2-280	plasma	100	3rdtrim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Inuit	17	GM	ND-150	13-23	plasma	52	3rdtrim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Denets/Metis	5.9	GM	ND-34	3.5-9.9	plasma	17	3rdtrim/delivery	2005-2007	Curren et al., 2015
USA, Alaska	Yup`ik, Inuit	15	GM		1.5-148	plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD; limit of detection; M, median; ND, non-detected

Table S6f. Worldwide comparisons of serum or plasma PCB 138 (µg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	PCB 138		95% CI	range	Material	n	Period	Year	References
Argentina	Ushuaia	6.3	GM	5.6-7.2	0.1-45	serum	199	2 days postpartum	2011	present study
	Salta	6.4	GM	5.8-7.0	0.2-172	serum	471	2 days postpartum	2011-2012	present study
Peru	Trujillo	6.5	AM	4.9-8.5		serum	59	delivery	2004-2005	Adetona et al., 2013
South Africa	Atlantic	3.6	GM	3.0-4.3	0.34-18	plasma	61		2008	Rollin et al., 2009
Korea	4 sites	4.6	M			serum	105	delivery	2011	Kim et al., 2013
Mexico	10 sites	2.4	GM	2.2-2.6		plasma	240	3rd trimester	2005-2006	Adlard, 2014
Canada	4 sites	3.7	GM	3.3-4.1		plasma	103	1st trimester	2005-2006	Adlard, 2014
Canada	10 sites	4.21	GM		ND-15	plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Caribbean	St. Vincent/Grenadines	8.5	GM	7.1-10		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Grenada	4.6	GM	3.9-5.5		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Belize	2.2	GM	1.8-2.6		serum	50	pregnancy	2008-2011	Forde et al., 2014
Spain	Catalonia, Sabadell	17	GM	16-17		serum	631	1st trimester	2004-2006	Ibarluzea et al., 2011
	Basque, Gipuzkoa	29	GM	28-31		serum	628	1st trimester	2006-2008	Ibarluzea et al., 2011
Italy	Brescia	35	GM	16-120		serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	15	GM		2.8-118	serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Faroe Islands		54	GM		3-383	plasma	500		2007-2009	AMAP 2015
Island	Reykjavik	15	GM		6.0-60	plasma	33	3rd trimester	2009	AMAP 2015
Russia	Murmansk Oblast	9.2	GM		1.0-48.2	plasma	50		2013-2014	AMAP 2015
Greenland	National, Inuit	29	GM		4.0-320	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
	East, Inuit	45	GM		4.8-180	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
Canada Arctic	Nunavik, Inuit	17	GM		<LOD-77	plasma	112		2012	AMAP 2015
Canada Arctic	Baffin, Inuit	15	GM	3.5-77	13-18	plasma	100	3rd trim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Inuit	8.6	GM	ND-99	6.5-11	plasma	52	3rd trim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Denets/Metis	3.1	GM	ND-13	2.1-4.7	plasma	17	3rd trim/delivery	2005-2007	Curren et al., 2015
USA, Alaska	Yup`ik, Inuit	9.1	GM		1.0-78	plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD, limit of detection; M, median; ND, non-detected

Table S6g. Worldwide comparisons of serum or plasma PCB 118 (µg/kg lipid) of pregnant or delivering women for the 2004-2014 period

Country	Region/area	PCB 118		95% CI	range	Material	n	Period	Year	References
Argentina	Ushuaia	3.1	GM	2.7-3.6	0.5-67	serum	199	2 days postpartum	2011	present study
	Salta	5.7	GM	5.2-6.3	0.6-163	serum	471	2 days postpartum	2011-2012	present study
Peru	Trujillo	2.8	AM	2.2-3.7		serum	59	delivery	2004-2005	Adetona et al., 2013
South Africa	Atlantic	1.5	GM	1.2-1.7	0.3-2.9	plasma	61		2008	Rollin et al., 2009
Korea (median)	4 sites	2.3	M			serum	105	delivery	2011	Kim et al., 2013
Mexico	10 sites	NA	GM			plasma	240	3rd trimester	2005-2006	Adlard et al., 2014
Canada	4 sites	2.2	GM	1.9-2.4		plasma	103	1st trimester	2005-2006	Adlardet al., 2014
Canada	10 sites	2.4	GM		ND-6.8	plasma	1935	1st trimester	2008-2011	Fisher et al., 2016
Caribbean	St. Vincent/Grenadines	2.9	GM	2.4-3.4		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Grenada	1.9	GM	1.6-2.2		serum	50	pregnancy	2008-2011	Forde et al., 2014
	Belize	NA	GM			serum	50	pregnancy	2008-2011	Forde et al., 2014
Italy	Brescia	11	GM	2-34		serum	70	delivery	2006	Bergonzi et al., 2009
Norway	Northern Norway	4.1	GM		1.0-38	serum	508	2nd trimester	2007-2009	Veyhe et al., 2015
Faroe Islands		15	GM		1-134	plasma	500		2007-2009	AMAP 2015
Island	Reykjavik	8.4	GM		4.7-18	plasma	33	3rd trimester	2009	AMAP 2015
Russia	Murmansk Oblast	26	GM		9.4-119	plasma	50		2013-2014	AMAP 2015
Greenland	National, Inuit	9.5	GM		1.5-100	plasma	194	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
	East, Inuit	17	GM		2.4-63	plasma	15	2nd trimester	2011-2013	AMAP 2015, Long et. al., 2015
Canada Arctic	Baffin, Inuit	6.2	GM	5.3-7.1	1.8-38	plasma	100	3rdtrim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Inuit	4.1	GM	3.1-5.3	ND-55	plasma	52	3rdtrim/delivery	2005-2007	Curren et al., 2015
	Inuvik, Denets/Metis	1.6	GM	1.2-2.2	ND-4.8	plasma	17	3rdtrim/delivery	2005-2007	Curren et al., 2015
USA, Alaska	Yup`ik, Inuit	3.4	GM		<LOD-28	plasma	156		2009-2012	AMAP 2015

AM, arithmetic mean; GM, geometric mean; LOD limit of detection; M, median; NA or ND, not reported due to high numbers of non-detected samples

ARTICLE 3

Drivers of maternal accumulation of organohalogen pollutants in Arctic areas (Chukotka, Russia) and 4,4'-DDT effects on the newborns

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Environmental International 124: 541-552 (2019)

DOI 10.1016/j.envint.2019.01.049

Environment International 124 (2019) 541–552

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Drivers of maternal accumulation of organohalogen pollutants in Arctic areas (Chukotka, Russia) and 4,4′-DDT effects on the newborns

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Arctic
Chukotka
Human biomonitoring
POPs
4,4′-DDT effects on newborns
Maternal serum
Organochlorine compounds

ABSTRACT

Background: One of the most worrying consequence of the production and use of persistent organohalogen pollutants (POPs) is the high accumulation in Arctic populations because of long-range transport. Study of the effects in these populations may illustrate human impacts that are difficult to assess in other locations with lower exposure to these compounds and more diverse pollutant influences.

Objective: We aimed to identify the main maternal characteristics influencing on the accumulation of these compounds and the effects on the newborns in a highly exposed Arctic population (Chukotka, Russia).

Methods: Organochlorine and organobromine compounds were analysed in maternal venous serum (n = 250). The study included data on residence, educational level, age, parity and body mass index (BMI) from self-reported questionnaires and measured anthropometric characteristics of newborns.

Results: Concentrations of β-hexachlorocyclohexanes, hexachlorobenzene, 4,4′-DDT and polychlorobiphenyls were high when compared with those generally found in adult populations later than year 2000. The polybromodiphenyl ethers were negligible. These POP concentrations were higher than in Alaska and Arctic Norway and similar to those in Canada. The Chukotka mothers living in inland areas showed significant lower concentrations than those living in the coast ($p < 0.001$) except for 4,4′-DDT. The population from the Chukotsky District, a specific coastal area, showed the highest concentrations. Residence was therefore a main concentration determinant ($p < 0.001$) followed by maternal age, and in some cases parity and BMI ($p < 0.05$). 4,4′-DDT showed an association with the anthropometric characteristics of the newborns ($p < 0.05$). Mothers with higher 4,4′-DDT concentrations had longer gestational ages and gave birth to infants with higher weight and length.

Conclusions: The maternal accumulation patterns of POPs were mainly related with residence. Most of these compounds were found in higher concentration in women living at coastal areas except 4,4′-DDE and 4,4′-DDT which were of inland origin. This last pesticide was the pollutant showing positive associations with gestational age and newborn's weight and length. To the best of our knowledge, this is the first study reporting statistically significant associations between maternal 4,4′-DDT exposure and anthropometric characteristics of the newborns.

1. Introduction

Persistent organic pollutants (POPs) include a large variety of toxic substances, such as hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), mirex, polychlorobiphenyls (PCBs), polybromodiphenyl eters (PBDEs) and dichlorodiphenyltrichloroethane (DDT) and its metabolites.

Many POPs are semi-volatile, stable to environmental degradation and may undergo long-range atmospheric transport, being found in areas where they have not been used or produced, like polar regions and high-mountains (Wania and Mackay, 1993; Arellano et al., 2014). These pollutants are lipophilic and have affinity for the adipose tissue of living organisms where they bioaccumulate (Hites, 2004; Corsolini et al., 2014; Mitchell et al., 2012). In parallel to bioaccumulation, they biomagnify through the food chain and are eventually ingested by humans (Johnson-Restrepo et al., 2005).

In 2001 these compounds were banned by the Stockholm Convention (Stockholm Convention, 2001) but human populations are

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https://doi.org/10.1016/j.envint.2019.01.049
Received 10 November 2018; Received in revised form 14 January 2019; Accepted 18 January 2019
Available online 25 January 2019

still exposed to them. Diet is the main POP exposure source among general population. Because of their lipophilicity, these compounds are mainly found in animal products including meat, fat, fish, dairy items and eggs (Junqué et al., 2017; Llobet et al., 2003; Martí-Cid et al., 2007). Arctic marine mammals accumulate high POP concentrations by ingestion from the food web (Braune et al., 2005; Hickie et al., 2005; Ikonomou and Addison, 2008; Kucklick et al., 2002). These animals are the major traditional food source for indigenous people because of the availability and high nutritional values of their meat (Sharma, 2010). Arctic populations therefore undergo significant exposure to these compounds despite their limited production or use in these areas.

Once ingested, POPs are able to cross the placenta leading to prenatal exposure of the foetus (Vizcaíno et al., 2014; Jeong et al., 2018). Exposure to POPs during pregnancy may have adverse impact on child development and health. In utero exposure has been associated with low fetal growth and premature delivery, neurocognitive deficit, obesity, lower respiratory tract infections and wheeze and hormonal disruptions (López-Espinosa et al., 2016; Grandjean and Landrigan, 2014; Gascón et al., 2017; Muscogiuri et al., 2017; Morales et al., 2012). The study of these compounds in venous maternal serum during pregnancy provides significant assessments on the accumulation rates in the newborns (Vizcaíno et al., 2014; Vafeiadi et al., 2014). Moreover, birth outcomes may show intermediate effects between prenatal toxic exposures and children's health problems later in life, hence the influence of environmental agents on birth outcomes must be investigated (Vafeiadi et al., 2014).

Previous studies showed extremely high levels of serum organochlorine compounds (OCs) in women from the Chukotka Peninsula (Russia; Fig. 1) (Sandanger et al., 2003; Anda et al., 2007). In this context, the present study is aimed to investigate the POP evolution in a Chukotka native population by analysis of serum samples from pregnant women living both in coastal and inland areas, to examine the dependence of maternal POP accumulation from a set of socio-demographic factors and to identify the effects of this accumulation on different birth outcomes such as gestational age, weight, length and head circumference.

2. Methods

2.1. Population and study design

Chukotka is an autonomous region (Autonomous Okrug; Fig. 1) also called Chukchi Okrug using the generic name of the inhabitants that is located in the far northeast of the Russian Federation (latitude: 64–69°N; longitude 162–173°E) and separated from Alaska by the Bering Strait. About 50% of the territory is located above the Arctic Circle. This region is divided in districts (Fig. 1).

Between 2014 and 2015, maternal venous blood was collected from women (n = 250) in the last week of pregnancy. The study also included a maternal questionnaire data for family history, life-style, behavioural risk factors, as well as potential nutritional, occupational and household sources of exposure to POPs following the one used for indigenous women residents of Chukotka in AMAP (2004). Maternal height and weight, and length, weight and head circumference of infants at birth were measured. Informed consent was requested from the participating mothers.

Patient recruitment was performed in the sequence in which they were admitted to the regional delivery department in the period from 20th August 2014 to 18th February 2015 on the basis of their voluntary consent to participate in the study. The exclusion criteria were as follows, refusal to give informed consent (2 persons), blood or plasma transfusion within the prior 72 h (1 person), bleeding disorders during pregnancy (1 person), taking in commonly known medications that have a negative impact on lipid levels such as antipsychotics, anticonvulsants or hormones (3 persons).

The study protocol and informed consent form were approved by the local Committee for Biomedical Ethics at the Northwestern State Medical University named after I. Mechnikov, St. Petersburg, dated 11.02.2014.

2.2. Analytical methods

The procedures for sample preparation and analysis have already been described elsewhere (Grimalt et al., 2010). Briefly, serum samples were placed into centrifuge tubes and the recovery standards 1,2,4,5-tetrabromobenzene (TBB) and PCB-209 were added. POP extraction and isolation were achieved by addition of *n*-hexane and H_2SO_4, vortex

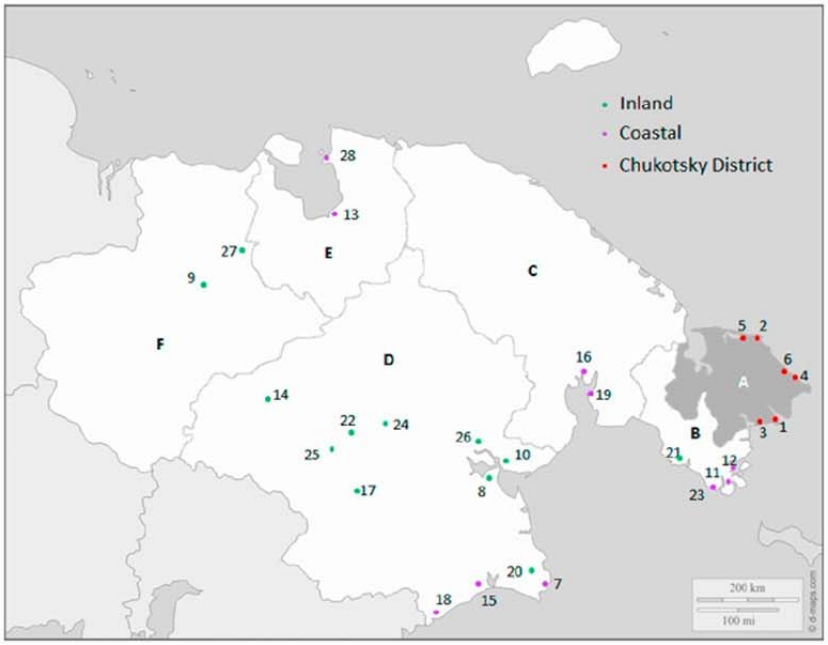

Fig. 1. Map of Chukotka showing the locations of the population participating in this study. Green dots inland areas. Purple and red dots coastal zones. Shadowed zone: Chukotsky District. Cities: 1: Lavrentiya, 2: Enurmino, 3: Lorino, 4: Uelen, 5: Neshkan, 6: Inchoun, 7: Beringovsky, 8: Anadyr, 9: Ceperveem, 10: Ugolnye Copi, 11: Providènіya, 12: Novoye Chaplino, 13: Ritkuchi, 14: Lamutskoye, 15: Meynypilgyno, 16: Egvekinot, 17: Vajegy, 18: Hatirka, 19: Konergino, 20: Alkatvaam, 21: Nunligran, 22: Snezhnoye, 23: Sireniki, 24: Ust-Belaya, 25: Markovo, 26: Kanchalan, 27: Bilibino, 28: Pevek. Districts: A: Chukotsky District, B: Providensky District, C: Lul'tinksy District, D: Anadyrsky District, E: Chaunsky District, F: Bilbinsky District. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixing and centrifugation. The supernatant *n*-hexane layer was aspirated into a second centrifuge tube. The acid layer was re-extracted two more times with *n*-hexane. All the *n*-hexane extracts were combined. This n-hexane solution was further purified by oxidation with concentrated H_2SO_4, vortex stirring and centrifugation. The acid was removed and H_2SO_4 was added again, followed by mixing and centrifuging once more. The supernatant organic phase was transferred to a conical bottomed, graduated tube and reduced to near dryness under a gentle stream of nitrogen. Then, the sample was transferred to gas chromatographic vials using three rinses of isooctane which were again reduced to dryness under a very gentle stream of nitrogen. Finally, they were dissolved with 100 μL of PCB-142 (internal standard) in isooctane.

Subsequent PBDEs analyses involved isooctane evaporation under a very gentle stream of nitrogen gas and dissolution with 20 μL of [3-^{13}C] BDE-209 and 30 μL of BDE-118 as internal standards (Vizcaíno et al., 2009).

Twenty OCs, pentachlorobenzene (PeCB), HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, PCB congeners 28, 52, 101, 118, 138, 153 and 180, 2,4′-DDD, 4,4′-DDD, 2,4′-DDE, 4,4′-DDE, 2,4′-DDT, 4,4′-DDT and mirex were quantified by gas chromatography and electron capture detection (GC-ECD, Agilent Technologies 7890A). The instrument was equipped with a HP-5MS capillary column (60 m length, 0.25 mm internal diameter, 0.25 μm film thickness; JW Scientific) protected with a retention gap. Two microliters were injected in splitless mode. Injector and detector temperatures were 250 °C and 320 °C, respectively. The oven temperature program started at 90 °C, held for 2 min, then it increased to 130 °C at 15 °C/min and to 290 °C at 4 °C/min with a final holding time of 15 min. Ultrapure helium was used as carrier gas. Nitrogen was the make-up gas. Compound quantification was performed as described elsewhere (Carrizo and Grimalt, 2009). Confirmation of the POP structures and checking for coelutions was performed with a GC (Agilent Technologies 7890N) coupled to a mass spectrometer (MS, Agilent Technologies 5975C) operating in negative chemical ionisation mode (GC-NICI-MS).

GC-NICI-MS was also used for identification and quantification of the PBDE congeners (17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190 and 209). The instrument was equipped with a DB-5 fused silica capillary column (15 m length, 0.25 mm I.D., 0.10 μm film thickness) protected with a retention gap. One microliter was injected.

The oven temperature program started at 90 °C which was kept for 1.5 min and continued by heating to 200 °C at 40 °C/min, a second increase up to 275 °C at 5 °C/min and a third to 300 °C at 40 °C/min. This temperature was held for 10 min and then increased to 310 °C at 10 °C/min with a final holding time of 2 min. Ammonia was used as reagent gas. Identification and quantification were performed by injection of PBDEs standard solutions (Vizcaíno et al., 2009).

2.3. Quality control

One procedural blank was included in each sample batch. Method detection limits were calculated from the average signals of the procedural blank levels plus three times the standard deviation. They ranged between 0.0014 and 0.027 ng/mL for the OCs and 0.0015–0.014 ng/mL for the brominated compounds. The limits of quantification were calculated from the averages of the procedural blanks plus five times the standard deviation ranging between 0.0020 and 0.038 ng/mL for the OCs and 0.0022 and 0.035 ng/mL for the PBDEs.

The methods were validated by analysis of proficiency testing materials obtained from the Artic Monitoring and Assessment Program (AMAP Ring Test, 2014). The IDAEA-CSIC laboratory participates regularly in the AMAP Ring Test Proficiency Program for POPs in human serum and the results were almost always within the acceptable range of ± 2SD of the consensus values, the causes of results out of this range were identified and solved, they did not refer to one or a few specific compounds.

2.4. Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). Statistics was focused on the compounds found above limit of detection in > 40% of the samples: HCB, α-HCH, β-HCH, 4,4′-DDE, 4,4′-DDT, PCB-118, PCB-138, PCB-153, PCB-180 and mirex. One-half of the limits of detection and limits of quantification were assigned to non-detected and non-quantified values, respectively.

Sample serum lipid content (TL) was calculated from the cholesterol (TC) and the triglyceride (Tg) concentrations (TL (g/L) = 2.27 * TC + Tg + 0.623); (Phillips et al., 1989).

Geometric means (GMs) and 95% confidence intervals (CI) were used for the descriptive analyses. Statistical differences between groups were tested for significance using Kruskall-Wallis rank test.

Multivariate curve resolution models using alternating least squares (MCR-ALS) (Tauler, 1995) and principal component analysis (PCA) were performed to assess the POP differences between the different areas of residence of the mothers. Before inclusion in the analysis, data were standardized and log transformed. The probability of the normal contour line from PCA was set at 69%.

Linear multivariate models with standardized variables were used to assess the dependences of maternal serum POP concentrations from age, body mass index (BMI), parity, smoking, education, residence and travel to other regions: $\log(OC) = \beta_1(Age) + \beta_2(BMI) + \beta_3(Parity) + \beta_4(Education) + \beta_5(Smoking) + \beta_6(Residence) + \beta_7(Travel) + \epsilon$. The obtained standard β coefficients were transformed into relative changes (%) in order to get better representation. For each variable, median serum concentrations by unit change (c), $(\exp(c * \beta) - 1) * 100$, and the corresponding confidence intervals, $(\exp(c * \beta \pm z_{1-\alpha/2} * SE(\beta)) - 1) * 100$, were calculated using β and standard errors (SE) from the multiregression analysis and *c* set as the difference between the first and third quartile (Barrera-Gómez and Basagaña, 2015).

The effects of maternal POPs on fetal growth outcomes, e.g. birth weight, length and head circumference, were assessed by linear multivariate models. The differences between POP concentrations between girls and boys were evaluated using Kruskall-Wallis rank test (Bravo et al., 2017). A sensitivity analysis was also performed for women with parity 1.

3. Results and discussion

3.1. Socio-demographic characteristics

The socio-demographic characteristics of the women included in the study and the anthropometric features of their newborns are shown in Table 1. Of the participating women, 146 were from inland cities (58%) and 104 (42%) were from coastal areas, 59 of these from Chukotsky District. Their average age was 27.8 years, with an overall age range between 15 and 44 years. According to pre-pregnancy BMI, 25% of the women were overweight or obese, while 68% had normal weight and only 7% were underweight. In 39% of the participant women, the actual newborn was the only child, in 32% it was the second child and in 29% they had 3 or more children. There was only one case of stillbirth. During pregnancy, 30 and 33% of the women smoked tobacco and consumed alcohol, respectively.

Of the infants, 51% were boys and 49% girls, the average weight and length were 3368 g and 52.5 cm, respectively. More detailed information about boys and girls can be found in Table 1. Gestational age average was 275 days (39.2 weeks), ranging from 165 to 348 days (23.6–49.7 weeks). Eighty-eight percent of the infants were born in the expected gestational age range (37–42 weeks), while 8% were preterm and 4% postmature. Almost all of them had values for head circumference in the normal range, 33.2–35.7 and 32.7–35.1 cm, for boys and girls respectively (WHO, 2018).

Table 1
Socio-demographic characteristics of studied population in Chukotka (n = 247).

	Participants n (%)
All women	250 (100)
Age (n = 247)	27.8 ± 7.1
BMI (n = 244)	
Underweight (< 18.5 kg/m^2)	17 (7)
Normal weight (18.5–25 kg/m^2)	165 (68)
Overweight (25–30 kg/m^2)	40 (16)
Obese (≥25 kg/m^2)	22 (9)
Parity (n = 247)	
1	97 (39)
2	79 (32)
≥3	71 (29)
Educational level (n = 247)	
Elemental or lower secondary	39 (16)
Secondary	72 (29)
Secondary special	76 (31)
High education	60 (24)
Area of residence (n = 250)	
Inland	146 (58)
Coastal	45 (18)
Chukotsky District (coastal)	59 (24)
Travel to other region (n = 241)	
Never	53 (22)
Once a year	123 (51)
1–3 times a year	65 (27)
Smoking (n = 247)	
Yes	75 (30)
No	172 (70)
Alcohol consumption (n = 246)	
Yes	83 (34)
No	163 (66)
Children	245 (100)
Gender (n = 243)	
Boys	125 (51)
Girls	118 (49)
Weight (g)	
Boys (n = 125)	3374 ± 652
Girls (n = 116)	3352 ± 549
Length (cm)	
Boys (n = 125)	52.6 ± 3.7
Girls (n = 116)	52.4 ± 3.6
Gestational age (n = 232)	
Preterm (< 37 weeks)	19 (8)
Normal (37–42 weeks)	204 (88)
Postmature (> 42 weeks)	9 (4)
Head circumference (cm)	
Boys (n = 125)	34.4 ± 2.1
Girls (n = 116)	34.4 ± 2.4

3.2. Distributions of organohalogenated compounds

4,4′-DDT and the PCB-138 and PCB-153 were found above limit of detection in all cases (Table 2). HCB, 4,4′-DDE, PCB118 and the α and β isomers of HCH were detected in > 90% of the mothers (94–99%). PCB180 was above limit of detection in 78% of the samples and mirex in 43%. The remaining pollutants were detected in < 40% of the mothers.

The most abundant POP was 4,4′-DDE, with a median of 121 ng/g lipid (Table 2), followed by β-HCH, HCB and PCB-153 (37.8, 29.3 and 24.6 ng/g lipid, respectively). Average 4,4′-DDE represented 92% of total DDTs, β-HCH was the most abundant isomer contributing 86% of total HCHs, and PCB-153 was the most abundant PCB congener representing 45% of the sum of PCBs, followed by PCB-138 (25%), PCB-118 (17%) and PCB-180 (10%).

None of the PBDEs congeners were found in > 40% of the samples. Only 2 of the 14 PBDEs, BDE153 and BDE190, were above limit of detection in 15% of the samples. The low detection of these compounds is not related to differences in LOD or LOQ of the method used in the present study (Vizcaíno et al., 2009) with other studies, e.g. Forde et al. (2014), Kalantzi et al. (2011). The lack of detectable concentrations of these compounds in a large number of samples probably reflects the use of PBDEs in comparison to the OCs which has involved delays in the long-range transport and distribution of the organobrominated pollutants. A similar contrast in the distribution of both types of POPs was observed in the environmental distribution of the High Tatra mountains where the OCs were showing a distribution dominated by long-range transport and temperature effects but the PBDEs were still not reflecting these trends because of the latter use (Gallego et al., 2007). In view of these low concentrations further analyses were only devoted to the OCs.

3.3. Differences between coastal and inland dwellers

All POP levels were higher in mothers from coastal than in inland sites (Fig. 2). The differences were statistically significant for HCB, β-HCH, PCB-118, PCB-138, PCB-153, PCB-180, mirex ($p < 0.001$) and 4,4′-DDE ($p < 0.01$).

The use of a multivariate curve resolution model using alternating least squares (MCR-ALS) method indicated that POPs data from the coastal group was composed of two subgroups, one showing more variability than the other. According to this information, further examination of the data showed one area of low POP variability, Chukotsky District, which was treated as a separate zone from the other coastal zones (Fig. 1).

Further insight on the significance of these areas was obtained from principal component analysis (PCA) of these POP concentrations (Fig. 3). The biplot of scores and loading of PC1 and PC2, which accounted for 69% of the total variance showed that PC1 was mainly influenced by all the studied compounds except 4,4′-DDE and 4,4′-DDT, which influenced PC2. The Chukotsky District samples (Fig. 3, in red) could be distinguished from the other two groups by higher concentrations of PCBs, HCB, mirex and HCHs.

As shown in Fig. 4, POP concentrations in mothers from the Chukotsky District (Fig. 1) were higher than those in mothers from the other districts. The differences were highest for the PCB congeners. Thus, the geometric average concentrations of PCB-138, PCB-153 and PCB-180 from the Chukotsky district were about 3.3–4.2 higher than the geometric averages of the whole maternal cohort of the present study (Table 3). The ratio between the GMs of the Chukotsky District and the other districts for PCB118 was 2.4. Similar results were obtained from comparison of the medians (Table 3). Two other compounds showing a strong contrast between the Chukotsky District and the whole Chukotka cohort were HCB and mirex, with geometric average ratios of 3.1 and 3.7, respectively (Table 3). In the case of β-HCH, the Chukotsky District/Chukotka cohort ratio was 2.6, significantly higher than 1. On the contrary, 4,4′-DDE and 4,4′-DDT showed negligible differences in these ratios, 1.2 and 1.1, respectively.

These results are consistent with those of a previous study (AMAP, 2004) in which the concentrations of some of these OCs were determined in mothers from the Chukotsky (n = 47), Anadyrsky (n = 39) and Lul'tinsky Districts (n = 5) and Anadyr Town (n = 12) (Fig. 1). The GMs of HCB, β-HCH, mirex and ΣPCBs in the former District, 1.6, 2.0, 0.1 and 3.8 ng/mL, respectively, were higher than those in the other two districts and Anadyr town, 0.5–0.6, 0.6–1.0, 0.01–0.03 and 0.8–1.5 ng/mL, respectively. Conversely, as found in the present study, the GM concentrations of 4,4′-DDE and 4,4′-DDT in Chukotsky District, 2.4 and 0.2 ng/mL, respectively, were not significantly different from those of the other districts, 1.2–2.2 and 0.2–0.4 ng/mL, respectively.

Linear multivariate models considering residence, either Chukotsky district, coastal, or inland, and maternal characteristics, e.g. age, parity, education, smoking, travel to other regions, afforded a better comparison of the main variables determining POP accumulation. In these models, the different residence categories were evaluated in pairs, Chukotsky District, coastal and inland, after taking into account the effect of the maternal variables (Fig. 5). The results showed that residence was one of the main determinants of POP accumulation, namely

Table 2
Serum POP concentrations (ng/g lipid and ng/mL) in the population of study.

	LD[a] (ng/mL)	LQ[a] (ng/mL)	DF[b] (%)	Lipid adjusted (ng/g lipid) (n = 246)				Non-adjusted (ng/mL) (n = 250)			
				GM	(95% CI)[c]	Median	Range	GM	(95% CI)[c]	Median	Range
PeCB	0.006	0.010	1	< LD		< LD	nd–2.1	< LD		< LD	nd–0.017
HCB	0.027	0.038	99	35	(31–40)	29	nd–850	0.28	(0.25–0.32)	0.29	nd–6.0
α-HCH	0.007	0.011	94	3.2	(2.9–3.5)	3.3	nd–100	0.025	(0.023–0.028)	0.027	nd–0.88
β-HCH	0.010	0.017	98	35	(30–41)	38	nd–660	0.28	(0.24–0.33)	0.29	nd–5.0
γ-HCH	0.013	0.020	38	< LQ		< LD	nd–13	< LQ		< LD	nd–0.091
δ-HCH	0.020	0.031	21	< LD		< LD	nd–93	< LD		< LD	nd–0.85
2,4′-DDD	0.007	0.012	14	< LD		< LD	nd–9.2	< LD		< LD	nd–0.077
4,4′-DDD	0.002	0.004	27	< LQ		< LD	nd–53	< LQ		< LD	nd–0.50
2,4′-DDE	0.013	0.020	7	< LD		< LD	nd–9.7	< LD		< LD	nd–0.067
4,4′-DDE	0.013	0.021	99	120	(110 − 130)	120	nd–1100	0.96	(0.86–1.1)	0.92	nd–5.4
2,4′-DDT	0.005	0.008	36	< LQ		< LD	nd–74	< LQ		< LD	nd–0.69
4,4′-DDT	0.005	0.008	100	9.0	(8.4–9.7)	8.5	2.0–67	0.25	(0.22–0.29)	0.065	0.014–0.51
Mirex	0.005	0.008	43	< LQ		< LD	nd–46	< LQ		< LD	nd–0.26
PCB-28	0.010	0.016	27	< LD		< LD	nd–36	< LD		< LD	nd–0.24
PCB-52	0.005	0.008	18	< LD		< LD	nd–750	< LD		< LD	nd–7.0
PCB-101	0.001	0.002	9	< LD		< LD	nd–8.0	< LD		< LD	nd–0.058
PCB-118	0.011	0.017	98	10	(9.0–11)	9.2	nd–1100	0.081	(0.071–0.091)	0.073	nd–9.7
PCB-138	0.002	0.003	100	17	(15–19)	14	1.3–440	0.13	(0.12–0.15)	0.12	0.013–4.1
PCB-153	0.007	0.012	100	31	(27–35)	25	3.9–880	0.25	(0.22–0.29)	0.20	0.032–8.2
PCB-180	0.011	0.018	78	5.4	(4.6–6.4)	5.4	nd–230	0.044	(0.037–0.052)	0.049	nd–2.2

[a] LD, LQ: Limit of Detection and Limit of Quantification.
[b] DF: Detection Frequency, % of samples above the limit of detection.
[c] GM (95%CI): Geometric mean with 95% confidence intervals.

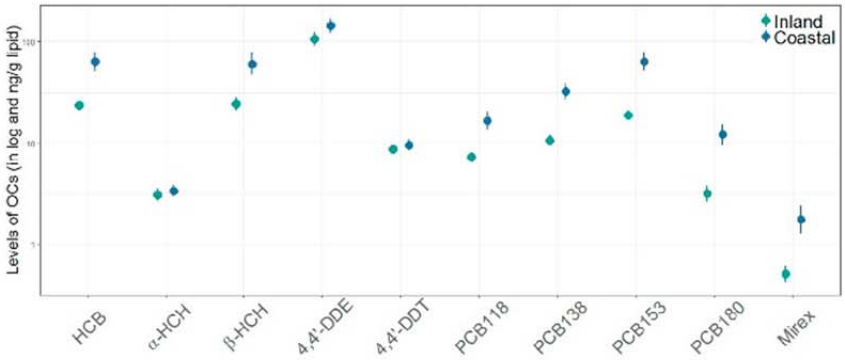

Fig. 2. Geometric means of the organochlorine compounds concentrations (ng/g lipid) in mothers from Chukotka living in inland or coastal areas. The vertical bars plot the 95% confidence intervals.

for PCBs, β-HCH and mirex.

These differences in POP concentrations may be explained by the diverse diets of coastal and inland populations. The former has a rich diet in marine mammals (whale, walrus, seal) as a food staple. These mammals are in the top of the food web being the highest bioaccumulators of long-range transported POPs to marine Arctic areas. The latter mainly consume reindeer meat and fish (Dudarev, 2012), involving a lower intake of marine sourced POPs. These diet differences between inland and coastal populations are even stronger when considering the Chukotsky District (Fig. 1) that is mainly populated by Chukchi or Yupik indigenous people whose economy is much more focused on traditional marine mammal hunting and reindeer herding (Pelyasov et al., 2017; ANSIPRA, 2018). The uniform distribution of DDT and its metabolites in coastal and inland populations is consistent with the past extensive use of this insecticide to protect reindeer skin against mosquito bites (AMAP, 2004) which overcomes possible influences related with long-range transport, including atmospheric inputs from China.

3.4. Comparison with other studies

In general, concentrations of β-HCH, HCB, 4,4′-DDT and PCBs in mothers of Chukotka are high when compared with other adult populations after year 2000 (Table 3). The concentrations from the mothers from Chukotsky District are even more prominent. Other pollutants such as PBDEs are found below limit of detection in 85% of the cases.

3.4.1. Comparison with other sites than Arctic populations

The concentrations of β-HCH in the present Chukotka study (median; 38 ng/g lipid) are higher than all cases previously reported except in a China study (median; 74 ng/g lipid; Table 3). The concentrations of HCB in Chukotka (median; 29 ng/g lipid) are higher than all these other cases compared except in China and Tunisia (75 and 39 ng/g lipid, respectively). However, the medians of the Chukotsky District for β-HCH and HCB (93 and 99 ng/g lipid, respectively) are higher than all previous cases (Table 3).

The distributions of PCBs in the blood serum of the Chukotka mothers are dominated by PCB-153 and PCB-138 and PCB-118 are the second and third most abundant congeners, respectively. This distribution is different from that reported in some constituents of the Russian dietary composition, e.g. butter, in which PCB-118 was the second most abundant congener (Polder et al., 2010). The difference is consistent with the predominant origin of the PCBs from marine food as consequence of the global long-range transport of these compounds. In

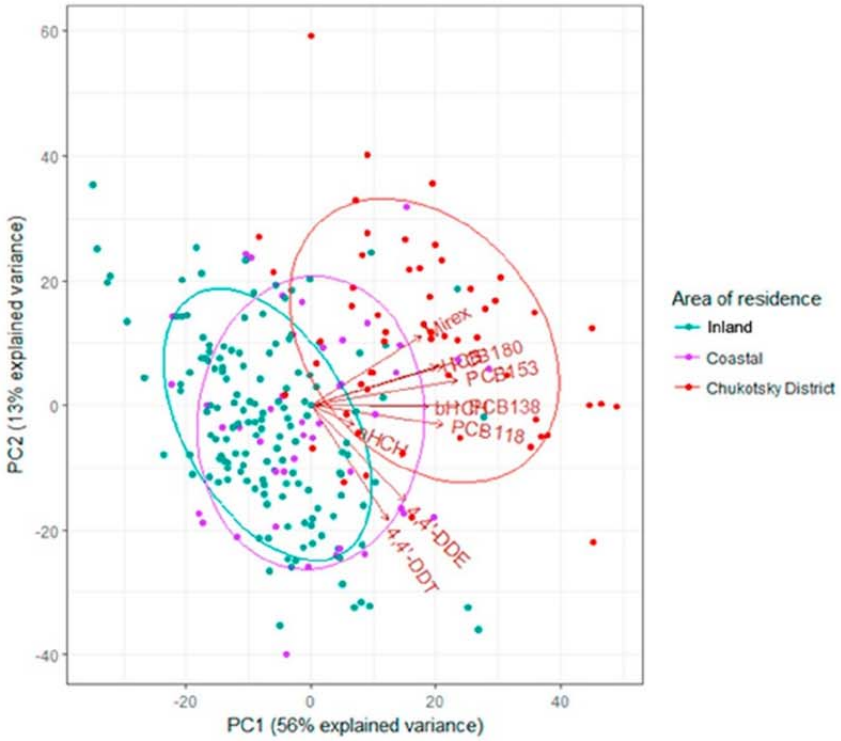

Fig. 3. Biplot of scores and loadings onto the first and second principal components for major organochlorine compounds by location.

fact, the PCB congener distributions of the mothers from Chukotsky District (Fig. 1) show even higher relative proportion of PCB-138 with respect PCB-118 and nearby the same proportion of PCB-118 and PCB-180, which reinforces the distinct composition of the marine sourced PCB mixtures in the mothers with higher marine mammal components in the diet. This group of mothers has the highest PCB composition when compared with previously reported literature data (Table 3). Comparison of the individual PCB congeners also shows that blood serum of these mothers contained the highest concentrations of PCB-118, PCB-138 and PCB-153 than in these previous studies (Table 3).

Comparison of the medians of the whole Chukotka mothers included in this study with previous maternal population studies from Canada (AMAP, 2015), Liege (Belgium), Sabadell (Catalonia) or Tunisia exhibit higher concentrations (Table 3). In these cases, comparison of the concentrations of some specific congeners such as PCB-118 is difficult because they were not often reported (Table 3).

Regarding DDT and its metabolites, 4,4′-DDT in the present study (median; 8.5 ng/g lipid) is higher than in all these previously mentioned studies except for those in Bizerte (Tunisia) and China (median; 24 and 17 ng/g lipid, respectively). 4,4′-DDE in Chukotka (median; 120 ng/g lipid) is higher than previous studies in populations from Argentina, South Africa, the Caribbean, Canada, Belgium or South Korea, similar to those found in Texas and Tunisia and lower than in China, Attika (Greece) and Sabadell (Spain) (median; 230, 270 and 110 ng/g lipid, respectively). The concentrations of the Chukotsky District (median; 160 ng/g lipid) are higher than in these previous cases except Attika (Greece) and China (Table 3).

3.4.2. Comparison with other Arctic populations

The Chukotka population, and more specifically that from Chukotsky District, are characterised for having higher levels when compared with other Arctic inhabitants (Table 3). Thus, all POPs are found in higher concentrations in Chukotsky District than in Alaska and Norway. The population of this District has also higher levels than in

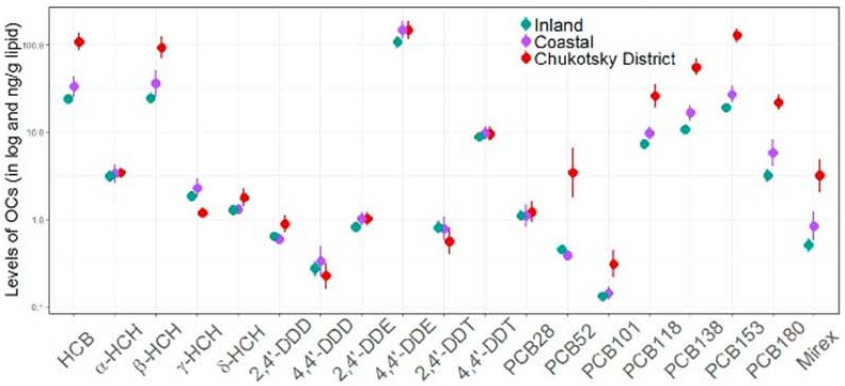

Fig. 4. Geometric means of the organochlorine compounds concentrations (ng/g lipid) in mothers from Chukotka living in inland and coastal areas and Chukotsky District. The vertical bars plot the 95% confidence intervals.

Table 3
Comparison of median concentrations of POPs in human serum from Chukotka with other populations (in ng/g lipid).

	Location	Year	N	PCB-118	PCB-138	PCB-153	PCB-180	β-HCH	4,4′-DDE	4,4′-DDT	HCB	Mirex	References
Arctic	Chukotka	2014–2015	250	9.2	14	25	5.4	38	120	8.5	29	< LD	Present study
				[10]	[17]	[31]	[5.4]	[35]	[120]	[9.0]	[35]	[0.83]	
	Chukotsky District	2014–2015	63	22	47	120	20	93	160	8.6	99	2.0	Present study
				[25]	[55]	[130]	[22]	[92]	[140]	[9.5]	[110]	[3.1]	
	Uelen city (Chukotsky D.)[a]	2014–2015	11	30	18	130	19	180	200	11	170	5.5	Present study
				[24]	[53]	[130]	[22]	[150]	[190]	[11]	[150]	[2.8]	
	Chukotka	2001–2002	48	[49]	[38]	[97]	[25]	[210]	[310]	[31]	[160]	–	Anda et al., 2007
	Uelen city (Chukotsky D.)[a]	2001–2002	50	140	250	640	160	520	560	36	200	29	Sandanger et al., 2003
				[110]	[210]	[540]	[150]	[410]	[520]	[34]	[170]	[27]	
	Alaska[b]	2009–2012	156	[3.4]	[9.1]	[15]	[5.4]	[3.6]	[83]	[2.5]	[16]	[2.3]	AMAP, 2015
	Canada[b]	2007–2008	485	[7.5]	[20]	[47]	[22]	[5.5]	[150]	[6.8]	[32]	–	AMAP, 2015
	Norway	2007–2009	508	[4.1]	[15]	[25]	[16]	–	[39]	–	[9.6]	–	Veyhe et al., 2015
Southern hemisphere	Ushuaia (Argentina)	2011–2012	199	3.3	5.8	8.1	1.0	6.8	27	3.0	8.3	–	Bravo et al., 2017
				[2.8]	[5.3]	[7.7]	[1. 6]	[5.1]	[33]	[2.7]	[8.7]	–	
	Salta (Argentina)	2011–2012	471	6.1	5.9	7.3	1.0	11	58	5.2	5.8	–	Bravo et al., 2017
				[4.8]	[5.3]	[6.8]	[1.6]	[7.8]	[67]	[5.7]	[5.2]	–	
	Bolivia	2013	200	–	–	–	–	–	200	–	–	–	Arrebola et al., 2016
	South Africa	2008	117	[1.5]	[3.6]	[3.2]	–	–	[29]	[7]	–	–	Channa et al., 2012
North America	Texas (USA)	2005–2009	461	2.5	5.3	8.2	6.5	1.7	110	2.1	8.0	1.6	Mumford et al., 2015
	Caribbean[c]	2008–2011	438	[1.8]	[3.8]	[7.0]	[4.1]	–	[70]	< LD	[3.6]	–	Forde et al., 2014
	Canada[b]	2007–2009	525	2.8	5.4	8.8	6.2	3.0	75	< LD	7.1	< LD	CHMS, 2010
Europe	Liege (Belgium)	2015	251	–	< LD	54	41	< LD	< LD	–	–	–	Pirard et al., 2018
	Attika (Greece)	2007	61	4.4	20	34	25	18	270	6.3	23	–	Kalantzi et al., 2011
	Sabadell (Spain)	2004–2006	631	–	[16]	[31]	[20]	[30]	[130]	–	[35]	–	Ibarluzea et al., 2011
North Africa	Bizerte (Tunisia)	2011–2012	113	< LD	24	49	32	9.5	120	24	39	–	Ben Hassine et al., 2014
	Tunis/Ariana (Tunisia)	2012	54	–	26	110	30	< LD	130	–	20	–	Artacho-Cordón et al., 2015
Asia	China	2010	81	–	–	–	–	74	230	17	75	0.23	Guo et al., 2014
	Korea	2011	105	2.3	4.6	9.0	–	7.6	57	5.2	9.5	–	Kim et al., 2013

In brackets geometric mean concentration

[a] See Fig. 1.

[b] Human plasma analysis instead of serum.

[c] Results from 10 different sites.

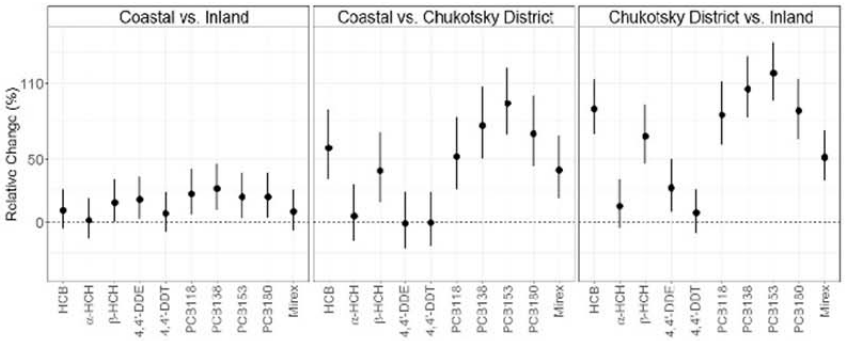

Fig. 5. Relative change (%) in median serum organohalogen concentrations by unit change calculated from the β coefficients and standard errors of the multi-regression analysis. The units of changes for each variable were set as the difference between the first and third quartile.

the Arctic Canada with the exception of 4,4′-DDE (median; 150 ng/g lipid).

The whole Chukotka population of the present study has higher POP concentrations than in Alaska and Norway, with the only exception of PCB180 (GM; 16 and 5.4 ng/g lipid, respectively). However, the concentrations in the Arctic Canada are close to those of the global Chukotka region. Thus, levels of PCB-138, PCB-153 and PCB-180 (GM; 20, 47 and 22 ng/g lipid, respectively) are found higher in the former than in the population of Chukotka (GM; 17, 31 and 5.4 ng/g lipid, respectively). The same is the case of 4,4′-DDE, with GM of 150 and 120 ng/g lipid in the Arctic Canada and Chukotka, respectively. The concentrations of all other POPs are higher in Chukotka than in Arctic Canada (Table 3).

In comparison with other Arctic sites, the mothers from Chukotka and Canada show similar concentrations which are higher than in other locations while Alaska has the populations with lowest concentrations.

Table 4
Results of the regression models showing effects of various determinants in blood serum (n = 226).

Compound	Variable	Std. β[a]	p	Compound	Variable	Std. β[a]	p
HCB	Age	0.083	0.21	PCB-118	Age	0.15	**0.040**
	BMI[b]	0.017	0.75		BMI[b]	0.096	0.11
	Parity	0.14	**0.031**		Parity	−0.013	0.85
	Education[c]	−0.053	0.44		Education[c]	−0.011	0.89
	Smoking[d]	0.082	0.19		Smoking[d]	0.015	0.82
	Residence[e]	0.38	**< 0.0001**		Residence[e]	0.41	**< 0.0001**
	Travel other region[f]	−0.20	**0.0018**		Travel other region[f]	−0.10	0.14
α-HCH	Age	0.026	0.75	PCB-138	Age	0.14	**0.038**
	BMI[b]	0.11	0.093		BMI[b]	0.034	0.55
	Parity	0.030	0.70		Parity	−0.074	0.25
	Education[c]	0.032	0.70		Education[c]	0.0013	0.98
	Smoking[d]	0.034	0.66		Smoking[d]	0.076	0.23
	Residence[e]	0.056	0.46		Residence[e]	0.50	**< 0.0001**
	Travel other region[f]	−0.053	0.51		Travel other region[f]	−0.12	0.057
β-HCH	Age	0.17	**0.015**	PCB-153	Age	0.067	0.30
	BMI[b]	0.21	**0.00053**		BMI[b]	0.025	0.64
	Parity	0.040	0.56		Parity	−0.025	0.69
	Education[c]	−0.0006	0.99		Education[c]	0.00034	0.99
	Smoking[d]	0.084	0.21		Smoking[d]	0.042	0.49
	Residence[e]	0.33	**< 0.0001**		Residence[e]	0.50	**< 0.0001**
	Travel other region[f]	−0.11	0.13		Travel other region[f]	−0.15	**0.015**
4,4′-DDE	Age	0.28	**0.00037**	PCB-180	Age	0.16	**0.019**
	BMI[b]	0.13	**0.050**		BMI[b]	0.020	0.72
	Parity	−0.23	**0.0024**		Parity	−0.011	0.87
	Education[c]	0.063	0.42		Education[c]	0.0024	0.97
	Smoking[d]	0.14	0.058		Smoking[d]	0.049	0.45
	Residence[e]	0.22	**0.0022**		Residence[e]	0.44	**< 0.0001**
	Travel other region[f]	0.0072	0.92		Travel other region[f]	−0.19	**0.0045**
4,4′-DDT	Age	0.25	**0.0016**	Mirex	Age	−0.024	0.72
	BMI[b]	0.18	**0.0064**		BMI[b]	0.055	0.33
	Parity	−0.18	**0.016**		Parity	0.14	**0.030**
	Education[c]	−0.068	0.39		Education[c]	0.031	0.66
	Smoking[d]	0.12	0.11		Smoking[d]	0.15	**0.023**
	Residence[e]	−0.065	0.37		Residence[e]	0.25	**< 0.0001**
	Travel other region[f]	−0.080	0.29		Travel other region[f]	−0.28	**< 0.0001**

Bold p values are statistically significant ($p < 0.05$).

[a] β coefficients of the multivariate regression models after standardizing all the variables.

[b] BMI: Body mass index.

[c] Elemental education as the reference level.

[d] Women who don't smoke as reference.

[e] Inland as reference category for residence.

[f] Women who never travel to other regions as reference category.

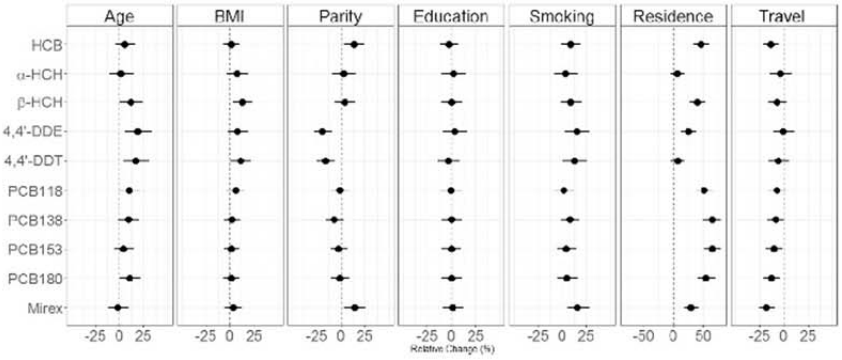

Fig. 6. Relative change (%) in median serum organohalogen concentrations by unit change calculated from the β coefficients and standard errors of the multi-regression analysis described in Table 3. The units of changes for each variable were set as the difference between the first and third quartile.

3.4.3. Comparison with previous studies in Chukotka

For a better comparison of the data from the present study with previous results three different entries have been calculated, the whole Chukotka cohort (n = 250), Chukotsky District (n = 63) and Uelen city (n = 11) (see Fig. 1). Comparison of the present study with the results found in the same area during 2001 and 2002 (Anda et al., 2007) shows a significant decrease, between 3 and 5 times (Table 3). This decrease is not observed for PCBs when these previous results are compared with those from the population of the Chukotsky District.

A decreasing trend is also observed in Chukotsky District when comparing the present and previous results from AMAP, 2004 maternal concentration. The GMs of HCB, β-HCH, ΣHCHs, 4,4′-DDE and ΣPCBs in plasma were 1.6, 2.0, 2.1, 2.4 and 3.8 ng/mL, respectively, whereas the present observations are 0.28, 0.29, 0.30, 0.96 and 0.50 ng/mL, respectively. 4,4′-DDT is the only compound not showing a decreasing trend between these two studies, 0.20 and 0.25 ng/mL, AMAP (2004) and the present study, respectively.

One previous study (Sandanger et al., 2003) was specifically performed in 2001–2002 in Uelen (Fig. 1). Comparison of the results from this study (n = 50) with the concentrations observed in the same area in our study (n = 11) show a clear decrease for all compounds, between 13% and 90% depending on the POP (Table 3).

These observed changes are consistent with the dietary changes in Chukotka. At the end of the 1980 years a "European" type of diet was adopted by most of the indigenous population under the age of 30 years (Kozlov, 2004). However, in 2002 still 76% of the Chukotka population declared preference for native food over European diet. Progressive introduction of this European diet may have led to a drop of OCs.

3.5. Associations between POPs in blood serum and maternal characteristics

Linear multivariate models of the maternal socio-demographic characteristics and POP concentrations provide a comprehensive description of the main maternal factors related with the concentrations of these pollutants (Table 4, Fig. 6). As mentioned above, area of residence is the main determinant for most POPs, e.g. HCB, β-HCH, mirex and PCB-118, PCB-138, PCB-153 and PCB-180 ($p < 0.001$ in all cases). These compounds show the highest β coefficients for residence among the determinant variables considered. 4,4′-DDE also displays a significant age dependence ($p < 0.001$) being residence the second highest β coefficient (Table 4).

Aside from residence, age is the main determinant for 4,4′-DDE ($p < 0.001$), 4,4′-DDT ($p < 0.01$) and the second highest for β-HCH, PCB-118, PCB-138 and PCB-180 ($p < 0.05$) showing a positive significant correlation (Table 4). This trend is consistent with increases in the concentrations of PCBs and organochlorine pesticides with age observed in other studies (Coakley et al., 2018; Bravo et al., 2017).

The third variable influencing the most on the POP concentrations is travel to other regions (Table 4). Women who never left Chukotka have significant higher concentrations of HCB ($p < 0.01$), PCB-153 ($p < 0.05$), PCB-180 ($p < 0.01$) and mirex ($p < 0.0001$) than those who spent time periods away from this area. This correlation is consistent with residence. Travelling to other regions likely involved dietary changes and lower exposure to POPs.

Parity is also a main determinant for HCB ($p < 0.05$), 4,4′-DDE ($p < 0.01$), 4,4′-DDT ($p < 0.05$) and mirex ($p < 0.05$). Higher values are associated with significantly lower concentrations of 4,4′-DDE and 4,4′-DDT. This trend is expected and found in other similar studies (Veyhe et al., 2015; Vizcaíno et al., 2014; Manaca et al., 2013). The positive correlation between parity and HCB concentrations is unexpected.

Higher body mass index involves higher statistically significant increases of 4,4′-DDT ($p < 0.01$), 4,4′-DDE ($p < 0.05$) and β-HCH ($p < 0.001$; Table 4; Fig. 6). Overweight does not lead to pollutant dilution when the main food sources ingested have these lipophilic pollutants in high concentrations. In these conditions consistent associations between higher BMI and higher pollutant concentrations are observed

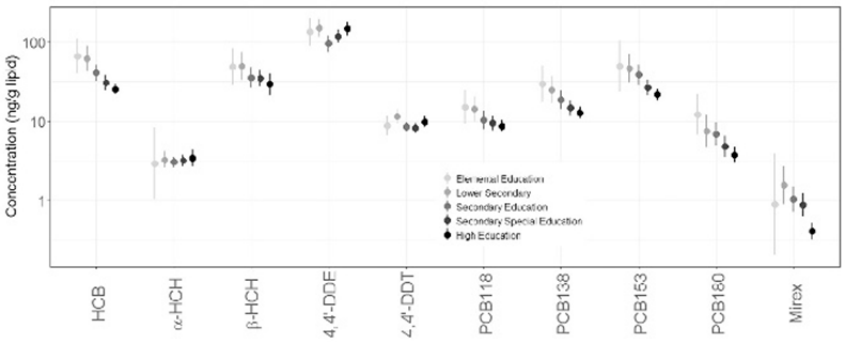

Fig. 7. Educational level plot of the geometric means and the 95% confidence intervals (ng/g lipid) of the organochlorine compounds concentrations in pregnant women.

concentrations is consistent with the observed metabolic effects for 4,4′-DDE.

However, these two compounds have different routes of interaction with human metabolism. Thus, in utero exposure to 4,4′-DDT has been described to decrease cognitive skills among pre-schoolers depending on genetic variability (Morales et al., 2008). These neurotoxic effects are specific of 4,4′-DDT (Ribas-Fito et al., 2006). In Chukotka, the observed influence of maternal 4,4′-DDT concentrations evidences a metabolic interaction of 4,4′-DDT already in the early life period which suggests that the deleterious effects identified in other cohorts will also occur at most advanced growth ages.

The other significant associations between higher maternal concentration of PCB-138 and lower gestational age and PCB-153 and size of the head circumference (Table 5) are consistent with other studies indicating effects of low-level environmental pollutants and fetal growth (Vafeiadi et al., 2014; Tatsuta et al., 2017). However, only these two congeners showed significant associations in the present cohort and these were related with different anthropometric characteristics which did not ground defined causal-effect relationships.

4. Conclusions

Women's residence was one of the main determinants for the PCBs, HCB, mirex and β-HCH, blood serum concentrations in pregnant women, involving higher concentrations in those living at the coast and particularly those from the Chukotsky District (Table 1). These differences can be explained by the different diets as people from the coastal areas have a more traditional diet, based on marine mammal hunting and reindeer herding than inland people. In this context, women from coastal areas who did not travel to other regions had highest concentrations of HCB, mirex, PCB-153 and PCB-180.

Other characteristics of the mothers such as age, are also main determinants of the concentrations of β-HCH, 4,4′-DDE, 4,4′-DDT and some PCB congeners.

The positive associations of maternal concentrations of 4,4′-DDT with higher birth weight, length and gestational age evidences that exposure to this compound has effectively an interaction with newborn metabolism. This pesticide is the POP showing a better defined influence on children's growth. The present study provides evidence of the influence of 4,4′-DDT on the anthropometric characteristics of the newborns for the first time. It is clear from the results of the present study that exposure to POPs, and particularly 4,4′-DDT, needs to be reduced for the benefit of the local inhabitants' health.

Conflicts of interests

The authors declare they have no actual or potential competing financial interests.

Acknowledgements

The authors are grateful to all women who participated in the study and the health staff who collected the samples. This study was partially funded by the Norwegian Ministry of Foreign Affairs through the Arctic Monitoring and Assessment Programme (AMAP). Additional funding from the EU projects NEUROSOME (H2020-MSCA-ITN-2017-766251), HEALS (FP7-ENV-2013-603946) and EDCMET (H2020-SC1-BHC-2018-SEP-210505108) is also acknowledged.

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3.4 Discussió dels resultats

En aquest capítol s'han estudiat una sèrie de contaminants orgànics persistents tant clorats com bromats. S'ha comprovat que malgrat porten molts d'anys prohibits i fora del mercat se segueixen trobant en mostres humanes, independentment del país d'origen.

Els resultats principals que s'han assolit s'han mostrat en forma de tres publicacions científiques. En aquesta secció es comentaran i discutiran els aspectes més rellevants per donar una visió global.

Concentracions de COPs en les poblacions d'estudi

Aquest estudi ha avaluat i descrit, per una banda, i per primera vegada, les concentracions de diferents contaminats orgànics persistents en sèrum d'una cohort de dones que acabaven de donar a llum, amb un número rellevant de participants, de l'hemisferi sud (Salta i Ushuaia a l'Argentina) i d'altra banda, sèrum d'una cohort de dones embarassades especialment exposada d'una regió de la Rússia àrtica (Chukotka).

Quant a pesticides organoclorats, com es pot observar a la Taula 2 de l'ARTICLE 1 i la Taula 2 de l'ARTICLE 3 de la secció de resultats, en les tres ciutats estudiades el compost s'ha trobat en concentracions més elevades ha estat el 4,4-DDE (amb medianes de 58, 27 i 120 ng/g lípid a Salta, Ushuaia i Chukotka, respectivament) amb freqüències de detecció altes (99-100%), seguit del β-HCH i el HCB, en aquest ordre a Salta i Chukotka i en ordre invers a Ushuaia. Respecte els contaminants organoclorats d'origen industrial, PCBs, el congènere més abundant ha estat el PCB-153 (medianes de 7,3, 8,1 i 25 ng/g lípid per Salta, Ushuaia i Chukotka, respectivament), seguit en tot tres casos, del PCB-138 i el PCB-118. En referència als retardants de flama bromats, PBDEs, a Rússia tant les freqüències de detecció com les concentracions trobades han

estat molt baixes. En el cas d'Argentina s'han trobat dos dels congèneres de PBDE en més del 75% de les mostres a Ushuaia i més del 58% a Salta, aquests són el BDE-153 i el BDE-209. El trobat en més abundància en totes dues ciutats va ser el BDE-209 (medianes de 3,5 i 2,8 ng/g lípid per Salta i Ushuaia, respectivament).

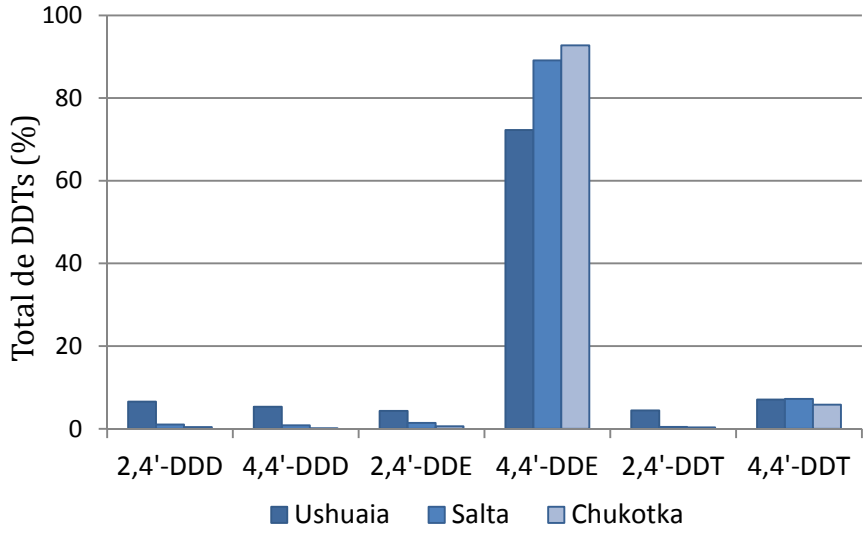

Figura 3.18 Contribució dels diferents isòmers del DDT a la suma molar total de DDTs en les poblacions de Ushuaia, Salta i Chukotka.

La distribució dels compostos de DDT en les tres poblacions estudiades esta dominada pel producte de degradació 4,4'-DDE (Figura 3.18). Això indica que no hi ha hagut exposició recent al seu compost d'origen (4,4'-DDT), la qual cosa és coherent amb la prohibició de DDT com a pesticida per a l'agricultura.

El perfil dels congèneres de PCB està dominat pel PCB-153, aquest contribueix a la suma de PCBs en un percentatge de 39, 32 i 45%, a Ushuaia, Salta i Chukotka, respectivament (Figura 3.19). Els altres congèneres majoritaris de PCB (PCB-118 i PCB-138) hi contribueixen en menor proporció, entre 17 i 25%, la resta dels PCBs (congèneres 28, 52, 101 i 180) estan contribuint a la suma total en menys de 5% (9% en el cas de Chukotka).

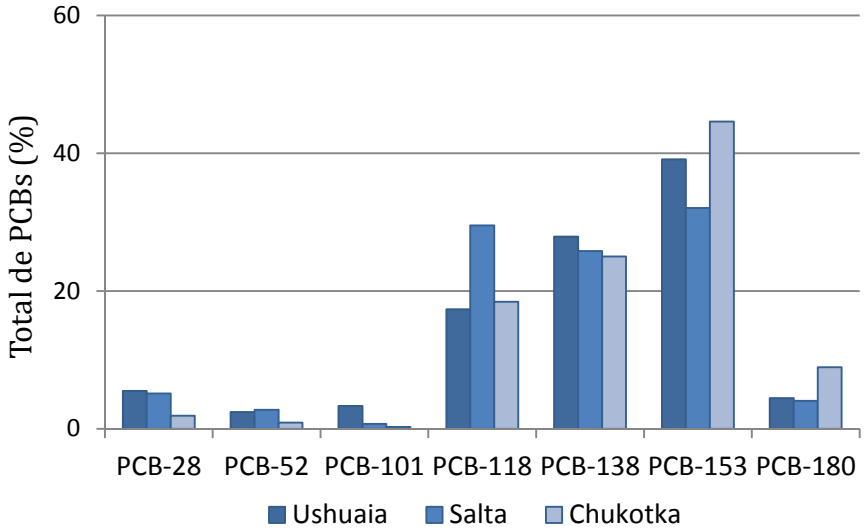

Figura 3.19 Contribució dels diferents congèneres de PCBs a la suma total de PCBs en les poblacions de Ushuaia, Salta i Chukotka.

En general, les concentracions de compostos organoclorats a Chukotka són més altes que a qualsevol de les dues ciutats d'Argentina. A l'ARTICLE 2 es comparen les concentracions de Salta i Ushuaia amb altres poblacions de dones embarassades o que acaben de donar a llum d'arreu del món. S'han afegit els resultats obtinguts en el treball de Chukotka en el context de l'ARTICLE 2 per ampliar la comparació.

A la Figura 3.20 es dona una visió global de les concentracions de 4,4'-DDE arreu del món. Es pot observar que les concentracions de 4,4'-DDE i el 4,4'-DDT d'aquest estudi són comparables amb les trobades en altres poblacions de l'Àrtic (ARTICLE 2; Fig. 2) com per exemple les de l'Illa de Baffin (Canadà), Nunavik (Canadà) i Groenlàndia (AMAP, 2015; Curren et al., 2015; Long et al., 2015) habitades pel grup ètnic Inuit, o les Illes Fèroe (AMAP, 2015). En relació amb altres regions del món, les concentracions són similars a les trobades a zones d'Europa central, Brescia (Itàlia) o Catalunya (Espanya) (Bergonzi et al., 2009; Ibarluzea et al., 2011), regions amb una alta activitat agrícola. Els nivells de 4,4'-DDE i 4,4-DDT de Chukotka són superats per poc per aquells trobats a

Bolívia, la Xina i el nord de Groenlàndia (Guo et al., 2014; AMAP, 2015; Arrebola et al., 2016) on les mitjanes geomètriques es troben entre 197 i 221 ng/g lípid. Les concentracions altes mesurades a Belize i Sud-àfrica no són sorprenents, perquè en aquests països està permesa la importació de DDT pel control dels mosquits com a part del seus programes de control de la malària (Channa et al., 2012; Forde et al., 2014).

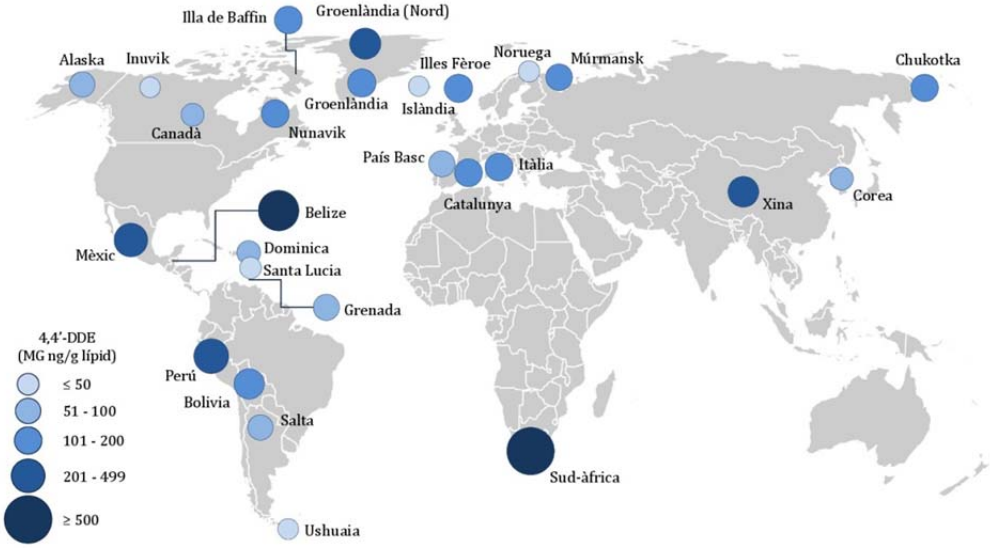

Figura 3.20 Mitjanes geomètriques (MG) de les concentracions de 4,4'-DDE (ng/g lípid) en dones embarassades o que acaben de donar a llum d'arreu del món. Dades disponibles al material suplementari de l'ARTICLE 2.

Pel que respecta als altres pesticides organoclorats comparats a l'ARTICLE 2, HCB i β-HCH, a Chukotka s'han trobat tots dos en una concentració de 35 ng/g lípid, similar a les trobades a Catalunya (Ibarluzea et al., 2011). En general, les concentracions de HCB són més altes que les de β-HCH a la resta de països (ARTICLE 2; Fig. 3), sent la primera comparable amb les mesurades al nord de Groenlàndia i el país Basc (Ibarluzea et al., 2011; AMAP, 2015). Respecte l'isòmer β dels HCH, Chukotka és un dels llocs amb nivells més alts, només superat per la Xina (68 ng/g lípid), lloc on s'ha utilitzat i produït extensivament pesticides organoclorats (Guo et al., 2014).

El congènere 153 dels PCBs és el més abundant en tots els estudis comparats. A Chukotka s'han trobat concentracions moderades (31 ng/g lípid) i similars a les d'altres regions de l'Àrtic, com l'Illa de Baffin, Nunavik (Canadà) i Islàndia (AMAP, 2015) i Catalunya (Ibarluzea et al., 2011). Tal com es mostra a la secció 4.2.3 de l'ARTICLE 2 les concentracions trobades tant a l'Argentina com a altres llocs de l'hemisferi sud i Àsia són bastant més baixes. Això es coherent amb el fet que la producció i l'ús de PCBs en aquestes zones han estat més limitats (Kallenborn et al., 2013).

Cal destacar, però, que la presa de mostra dels estudis inclosos a la comparació de l'ARTICLE 2 no es va realitzar en el mateix moment, sent l'estudi de Chukotka (ARTICLE 3) un dels més actuals. Estudis realitzats en diferents anys en un mateix lloc (Abass et al., 2018; Bravo et al., 2019), demostren que la prohibició d'aquests contaminants ha ajudat a que poc a poc amb el temps les concentracions altes que es trobaven a principis de l'any 2000 hagin anat disminuint. Per tant, s'hauria d'esperar que les concentracions d'alguns dels llocs que eren similars o superaven per poc a Chukotka, en un estudi realitzat el mateix any (2014-2015), es trobessin nivells més baixos o similars, respectivament.

Influència de l'edat, l'índex de massa corporal i la paritat en l'acumulació de COPs

La càrrega de contaminants en el cos d'una persona no depèn només de l'exposició a la que s'està sotmès. Als ARTICLES 1 i 3 s'han avaluat els patrons d'acumulació de COPs considerant la influència de l'edat, l'índex de massa corporal (IMC) i la paritat, entre d'altres.

El paper de l'edat

Els estudis amb la població de les dues ciutats d'Argentina i Chukotka presenten una associació molt forta amb l'edat en relació a l'acumulació de COPs.

Com s'ha discutit als ARTICLES 1 i 3, les concentracions dels OCs, en general, augmenten amb l'edat. A la Figura 3.21 està representada la concentració d'alguns dels compostos organoclorats més rellevants en funció de l'edat, ajustat per l'IMC, la paritat i el país. Aquesta associació és coneguda i està extensament recolzada a la literatura en molts tipus de poblacions (Porta et al., 2008; Rawn et al., 2012; Fromme et al., 2016; Eguchi et al., 2017; Thomas et al., 2017; Coakley et al., 2018).

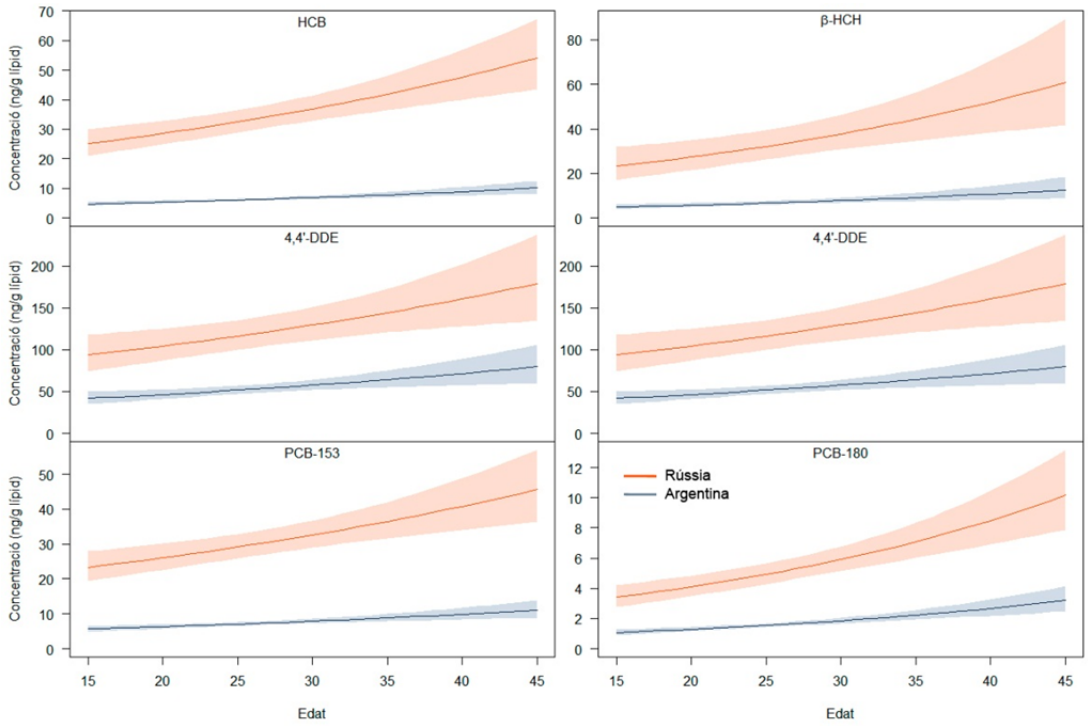

Figura 3.21 Sèrie de gràfics on es mostra l'acumulació de diferents compostos organoclorats segons l'edat i el país, ajustat per paritat i IMC. Rússia esta representada en taronja i Argentina en blau.

Aquest mateix patró s'observa tant en poblacions poc exposades, a les dues poblacions Argentines, com en poblacions que viuen en condicions d'alta exposició, com la de Chukotka a Rússia (Figura 3.21). Tot i que la relació es veu

millor quan s'analitza un marge ample d'edats (des de la infància fins a la vellesa), com que l'edat és un dels principals determinants que expliquen la concentració de compostos organoclorats, també es veu aquesta tendència en el rang de treball dels ARTICLES 1 i 3, on les voluntàries, dones embarassades o que acaben de donar a llum, tenen entre 15 i 45 anys.

A diferència dels compostos organoclorats, alguns estudis anteriors no observen cap relació entre les concentracions de PBDEs i l'edat (Zota et al., 2008; Antignac et al., 2009), mentre que d'altres mostren una tendència inversa d'alguns congèneres de PBDEs amb l'edat (Sjodin et al., 2008; Toms et al., 2009; Gari i Grimalt, 2013). Encara que els dos tipus de compostos (OCs i PBDEs) tenen propietats fisicoquímiques similars una de les hipòtesis que expliquen la divergència en els patrons d'acumulació és històrica: els compostos organoclorats es van començar a sintetitzar i utilitzar als anys 30, mentre que els PBDEs no es van comercialitzar fins la dècada dels 80, en conseqüència els organoclorats porten més temps alliberats al medi ambient.

En els estudis realitzats en aquesta tesi tampoc s'han trobat relacions significatives entre les concentracions de PBDEs i l'edat. Aquests compostos es troben en concentracions molt baixes en ambdós països, sovint per sota del límit de detecció, tant Argentina com Rússia són països on històricament no s'han fet servir els PBDEs.

En algunes ocasions hi ha estudis que desagreguen l'edat per any o dècada de naixement, d'aquesta manera les diferències es fan més evidents. Els individus que han nascut abans han estat exposats durant més temps que els més joves, que a més de portar menys anys exposats van néixer en un moment en què, probablement, la majoria dels compostos estudiats ja estaven prohibits o restringits. En els estudis realitzats en aquesta tesi no s'han trobat aquestes relacions.

Una de les limitacions dels estudis de poblacions de dones embarassades és que es treballa amb dones en edat fèrtil, per tant, respecte a un estudi amb població general, el marge d'edat d'estudi es redueix bastant, tot donant lloc a què algunes tendències que s'observen en població general, com l'associació amb PBDEs o les diferències entre dones nascudes en diferents anys o dècades, no s'observin en una població de dones embarassades.

En resum, una de les conseqüències de la persistència dels COPs és l'existència d'una correlació positiva amb l'edat, que, tot i no ser la única variable de la qual depèn la càrrega de COPs en el cos, és una de les més importants.

L'índex de massa corporal i l'acumulació de COPs

La població estudiada d'Argentina mostra diferents patrons d'acumulació de COPs en relació amb l'índex de massa corporal (ARTICLE 1; Fig. 2). A continuació es discuteix aquesta relació tenint en compte tant les mostres de l'estudi d'Argentina com de Rússia.

Les concentracions dels pesticides organoclorats tendeixen a augmentar a major IMC (Figura 3.22), les dones amb un índex de massa corporal superior tenen nivells de β-HCH, 4,4'-DDE, 4,4'-DDT i PCB-118 significativament més alts que les que tenen un IMC inferior. El patró que segueixen els PCBs segons l'IMC no és regular. Mentre que les concentracions de PCB-118 augmenten amb el índex de massa corporal, els nivells dels congèneres 138, 153 i 180, disminueixen amb l'IMC, tot i que no tenen significació estadística.

En el cas dels PBDEs no s'ha trobat cap relació significativa amb l'IMC per cap dels congèneres. Com s'ha comentat amb anterioritat aquesta manca de relacions pot ser deguda a la freqüència de detecció.

Els resultats de les poblacions estudiades en aquesta memòria de tesi són coherents amb els d'altres casos.(Porta et al., 2010; Gari i Grimalt, 2013).

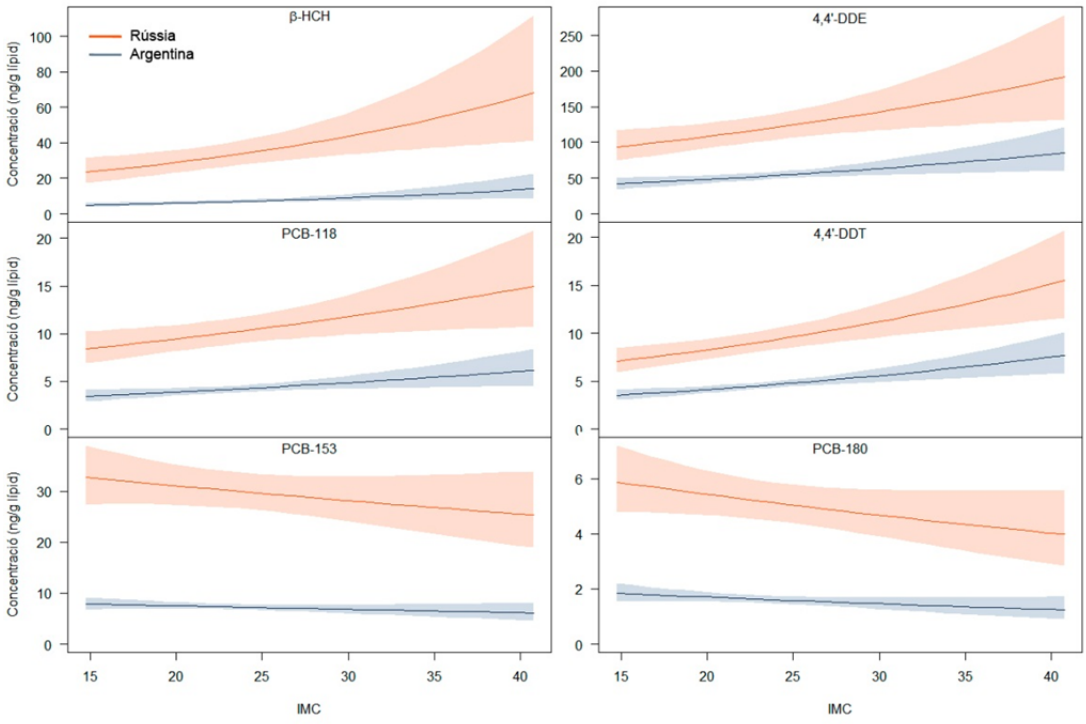

Figura 3.22 Sèrie de gràfics on es mostra l'acumulació de diferents compostos organoclorats segons l'IMC i el país, ajustat per edat i paritat. Rússia esta representada en taronja i Argentina en blau.

La paritat, una via de desintoxicació

Les cohorts utilitzades per realitzar els estudis d'aquesta tesi estan formades únicament per dones embarassades (ARTICLES 1 a 3). En aquests casos, a més de l'edat, la paritat és un dels factors més importants per explicar els patrons d'acumulació. S'entén com a paritat el número de nens vius que ha tingut una dona.

És sabut que hi ha una transferència de COPs de mares a fills durant l'embaràs, a través de la placenta, i durant la lactància a través de la llet materna (Vizcaino et al., 2014; Gascon et al., 2015; Lopez-Espinosa et al., 2015). Com es pot observar a la Figura 3.23, degut a aquesta transferència, les concentracions de la majoria de compostos disminueixen amb el número de fills, sent aquestes tendències significatives per l'HCB, el β-HCH, el 4,4'-DDE i els congèneres 118, 138, 153, 180 de PCBs.

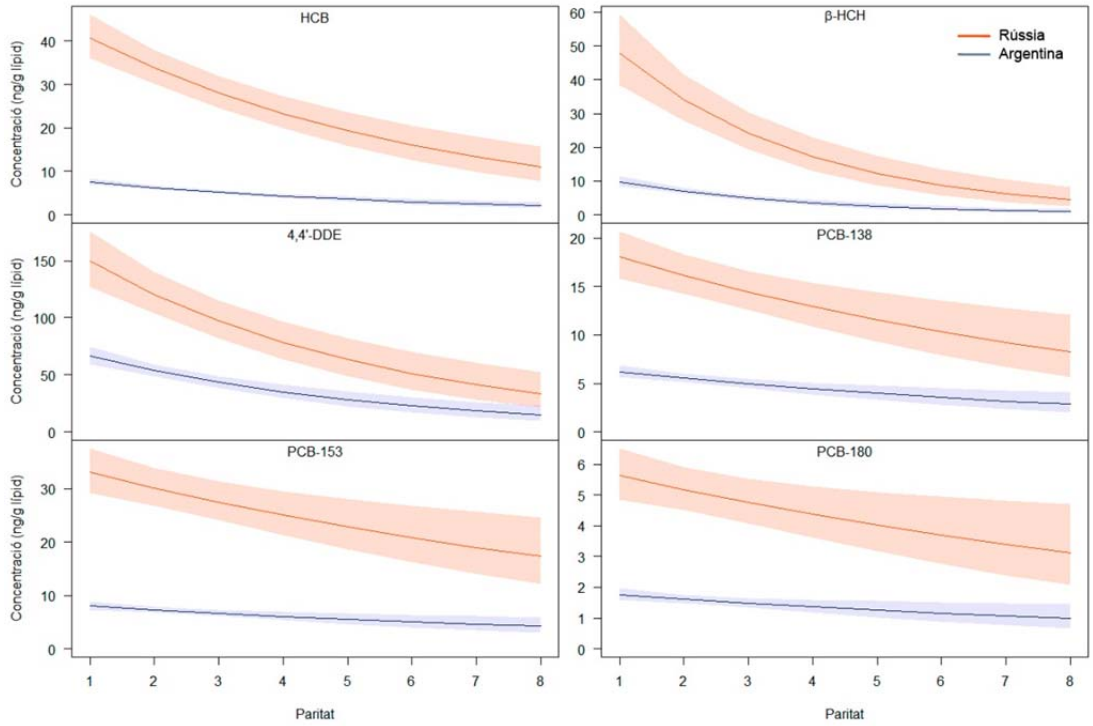

Figura 3.23 Efecte ajustat de la paritat amb les concentracions de COPs. Cada grup de valors representa un model de regressió multivariant ajustat per edat, IMC i país.

La paritat sovint està relacionada amb l'edat, és previsible que dones en edat més avançada hagin tingut més nens, per tant és importat avaluar els dos efectes en conjunt, tot realitzant anàlisis multivariants.

Influència d'altres factors sociodemogràfics en l'acumulació de COPs

A més dels factors intrínsecs de cada dona existeixen altres determinants que poden influenciar en l'exposició de COPs (Lewin et al., 2017). Per exemple a l'ARTICLE 1 i 2 s'ha avaluat com afecta la residència o el lloc de naixement i a l'ARTICLE 3 s'ha vist que viatjar durant un període més o menys llarg a un altre indret pot tenir un paper important en l'acumulació de COPs.

Lloc de naixement

A la secció "Concentracions de COPs en les poblacions d'estudi" s'ha vist que a Chukotka les concentracions són més altes que a qualsevol de les dues poblacions Argentines. Un cop s'han ajustat aquestes concentracions per edat, paritat i IMC aquestes diferències són més evidents i són estadísticament significatives per a tots els compostos excepte el PCB-153 (Figures 3.4-3.6).

Això es mostra a la Figura 3.24 que és equivalent a la Figura 3.21 però ara s'ha representat per separat les dues poblacions d'Argentina (Salta i Ushuaia). Es pot observar que generalment a Salta les concentracions són superiors que a Ushuaia excepte en el cas de l'HCB. Aquestes diferències es discuteixen a la secció "Dependència de la latitud".

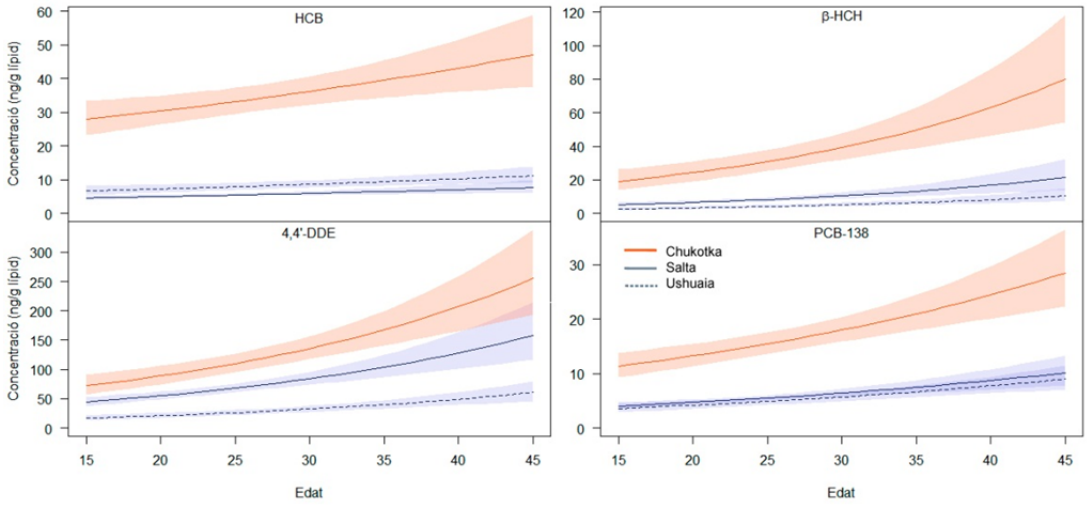

Figura 3.24 Efecte ajustat de l'edat amb les concentracions de COPs estratificat per província/districte. Cada grup de valors representa un model de regressió multivariant ajustat per paritat, IMC i província/districte.

A la Fig. 1 i la Taula 2 de l'ARTICLE 2 es mostren les concentracions ajustades per edat i paritat així com la significança estadística de les diferències en les concentracions dels OCs més rellevants en les dues poblacions argentines estratificades segons si han nascut a les àrees d'estudi o si són immigrants. No s'observen diferències significatives en cap de les dues ciutats segons si són natives o immigrants. Aquest resultat és coherent amb altres estudis realitzats

on es troben diferències significatives en quant a immigració entre països però no entre regions d'un mateix país (Vizcaino et al., 2010; Porta et al., 2012).

Lloc de residència

Tant a l'ARTICLE 1 com al 3 s'han estratificat les voluntàries segons el seu lloc de residència, a l'ARTICLE 1 segons si vivien en zones urbanes, rurals o industrials i a l'ARTICLE 3 segons si vivien a la costa o a l'interior.

Per una banda, a Salta (Argentina) es veu clarament com a les zones rurals i semi-urbanes (ARTICLE 1; Fig. 2) les dones acumulen més pesticides, especialment 4,4'-DDT i 4,4'-DDE, com a conseqüència de l'alta activitat agrícola de la zona. En quant als PCBs, contaminants d'origen industrial, la seva major aportació es troba en les zones urbanes de Salta.

Per l'altra banda, les dones residents en ciutats costaneres de Chukotka tenen concentracions significativament més elevades que aquelles que residien a l'interior (ARTICLE 3; Fig. 4), fet atribuïble a la dieta diferent entre les poblacions de la costa (basada en peix i grans mamífers aquàtics) i les poblacions de l'interior que s'alimenten majoritàriament de carn de caça.

Com s'ha vist a l'ARTICLE 3 no només importa el lloc on es resideix habitualment sino també si es passen temporades en llocs diferents. Les dones que en el moment de l'estudi no havien abandonat Chukotka en cap moment presenten concentracions significativament més altes d'HCB, alguns PCBs i mirex. Aquests viatges fora de Chukotka poden representar canvis en la dieta i una exposició més baixa a COPs.

Altres factors sociodemogràfics

Existeixen altres factors sociodemogràfics que poden afectar l'acumulació de COPs com són el nivell educatiu o la classe social. A continuació es comenten

breument, encara que en els estudis realitzats en aquesta tesi no s'han trobat associacions significatives.

El nivell educatiu i la classe social són indicadors intrínsecament relacionats ja perquè generalment els individus amb una educació superior poden arribar a un estatus social superior.

Alguns estudis han trobat que els individus amb un nivell educacional superior també tenen concentracions més altes de COPs (Vizcaino et al., 2010; Vrijheid et al., 2012; Fisher et al., 2016). Una possible explicació recau en els hàbits alimentaris ja que aquestes solen tenir una dieta més equilibrada amb una ingesta elevada de peix (Darmon i Drewnowski, 2008). Com s'ha descrit anteriorment la dieta és una de les rutes d'exposició més important i el consum de peix alt sol estar associat a nivells alts de COPs (Knutsen et al., 2011).

Efectes dels OCs en les dones embarassades i els seus nounats

S'han valorat els efectes negatius d'alguns compostos organoclorats sobre la salut de les dones embarassades de Chukotka i els seus fills. Concretament s'ha investigat l'associació que pot existir entre els nivells d'OCs i l'edat gestacional del nadó i la relació amb les diferents mesures antropomètriques del nounat (pes, longitud i perímetre cranial).

Mitjançant l'anàlisi multivariant s'han observat associacions significatives d'alguns dels contaminants estudiats (ARTICLE 3; Table 5) i l'edat gestacional després d'ajustar els models per edat de la mare, paritat, consum d'alcohol i tabac i pes i longitud del nadó. Els resultats suggereixen que existeix una associació positiva entre les concentracions de 4,4'-DDT i PCB-138 i l'edat gestacional, aquests resultats coincideixen amb els trobats en altres estudis (Vafeiadi et al., 2014; Tatsuta et al., 2017).

Respecte les mesures antropomètriques dels nadons també s'ha trobat una relació entre el 4,4'-DDT i el pes i longitud al néixer, i l'HCB i el PCB-153 amb la perímetre cranial. A la secció 3.6 de l'ARTICLE 3 es discuteix en detall aquestes associacions.

Es va estudiar la possible existència d'altres relacions, com per exemple el tipus de part (cesària o vaginal), malformacions en el fetus o complicacions durant l'embaràs. En el primer cas no es va trobar cap associació. En el cas de les malformacions en el fetus i les complicacions durant l'embaràs no es van poder realitzar els models per falta d'individus, ja que gairebé no hi havia casos de malformacions i pràcticament totes les dones havien passat un embaràs sense complicacions.

Dependència de la latitud

La Figura 3.25 mostra una comparació inicial entre la mitjana geomètrica de les concentracions de compostos organoclorats trobades entre les poblacions de Salta i Usuahia.

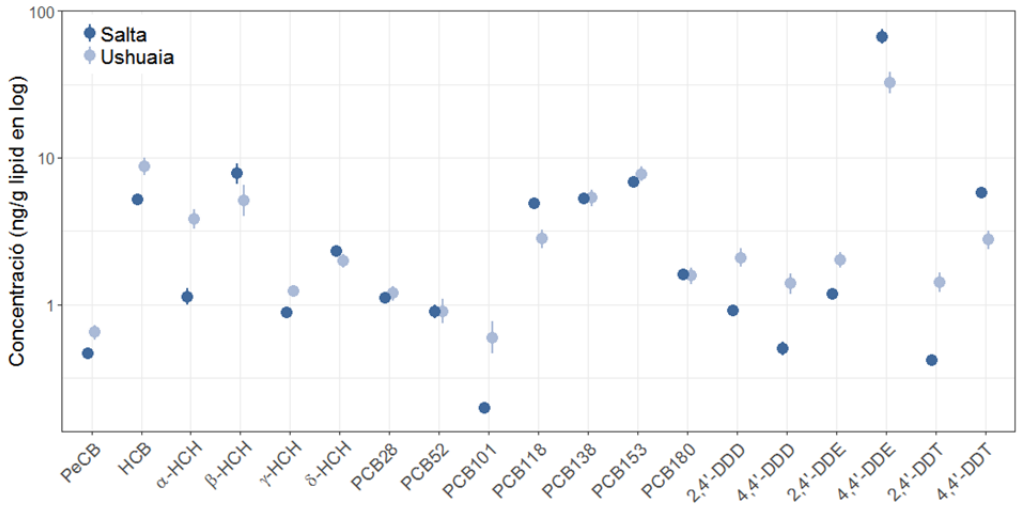

Figura 3.25 Mitjanes geomètriques de les concentracions de compostos organoclorats en mares de Salta i Ushuaia (ng/g lipd). Les barres verticals corresponen al interval de confiança del 95%.

Per avaluar les diferències entre les dues ciutats s'han realitzat models de regressió multivariant ajustats per les variables abans descrites (edat, IMC, paritat i residència). Utilitzant els nivells d'Ushuaia com a referència, els coeficients són positius i estadísticament significants ($p<0,05$) pel PeCB, HCB, α-HCH, γ-HCH i PCB101. Això indica que aquests contaminants es troben principalment a Ushuaia, mentre que el β-HCH, els congèneres 118, 138 i 180 de PCBs, el 4,4'-DDT i el seu principal metabòlit 4,4'-DDE són més abundants a Salta. Amb excepció del β-HCH, els compostos més abundants d'Ushuaia corresponen als més volàtils d'aquests estudi, mentre que els de Salta són els menys volàtils.

Entre Salta i Ushuaia hi ha fins a 34° de diferència en latitud que es tradueixen en un clima molt diferent, càlid a Salta i fred a Ushuaia. Aquests resultats són coherents amb el mecanisme de la desti·lació global, així part dels compostos que es troben a Ushuaia han arribat allà transportats des de zones més càlides. En relació al β-HCH es pot pensar que la seva concentració pot estar influenciada per altres determinants o fonts directes d'emissió en llocs propers a la zona d'estudi perquè pot reflectir-ne l'úsagrícola o ramader contra els insectes.

3.5 Conclusions

En aquest capítol s'han explorat els patrons d'acumulació de certs COPs en dones embarassades, mitjançant models multivariants de les distribucions de concentracions mesurades. A continuació es resumeixen les conclusions més importants.

Nivells de COPs a l'Argentina i Rússia. Totes les participants dels dos estudis realitzats mostren nivells detectables d'algun dels COPs analitzats. Això mostra l'impacte d'aquests compostos i la seva persistència en el medi ambient que afecta a la població humana fins i tot després d'haver estat prohibits o restringits durant anys.

Les concentracions de compostos organoclorats en les poblacions d'Argentina i Rússia són equivalents les descrites en altres poblacions d'arreu del món. A Rússia es troben valors més alts que a l'Argentina. El 4,4'-DDE (metabòlit de degradació del DDT) és el més detectat i més abundant en les tres ciutats, així com el més dominant d'entre tots els metabòlits de DDT. El predomini del 4,4'-DDE sobre el 4,4'-DDT és coherent amb la prohibició del DDT a la dècada dels 70 i per tant, només es troben els seus metabòlits resultants. Els compostos HCB i β-HCH també es troben en concentracions altes tal com es propi de països on històricament s'han utilitzat grans quantitats de pesticides. Respecte els PCBs, el més abundant en el congènere 153, i en general, a l'Argentina les concentracions són baixes perquè la seva producció i ús han estat més limitats, mentre que a Chukotka les concentracions són moderades, similars a les trobades en altres regions de l'Àrtic. Aquests nivells, en part, es poden explicar per l'alt consum de peix i grans mamífers. Respecte als PBDEs, en tots dos països s'han detectat poc i trobat en concentracions molt baixes. Aquests resultats són coherents amb la utilització baixa d'aquests compostos en aquestes regions.

Influència de l'edat, la paritat i l'IMC en l'acumulació de COPs. Els estudis realitzats confirmen que les concentracions d'organoclorats augmenten amb l'edat. A més a més, existeix una transferència de COPs de mares a fills a través de la placenta. Això fa que les concentracions de la majoria dels compostos organoclorats estudiats en les mares disminueixin amb el número de fills. L'edat i la paritat són dos determinants forts en l'estudi d'acumulació de compostos organoclorats.

Per altra banca, mentre que les concentracions del pesticides organoclorats tendeixen a augmentar amb l'índex de massa corporal, el patró que segueixen els PCBs no és regular, per uns augmenta (PCB-118) i per altres disminueix (PCBs 138, 153 i 180).

Degut a les concentracions baixes de PBDEs no s'han trobat associacions significatives amb l'edat, la paritat o l'IMC.

Influència de la residència en l'acumulació de COPs. S'ha demostrat que el lloc de residència té un paper molt important en l'exposició a compostos organoclorats. Les diferències no només són clares entre països, a Rússia les concentracions són més altes que a l'Argentina, sinó que també mostren una dependència segons si viuen en zones rurals o urbanes (Argentina) o si viuen a la costa o l'interior (Chukotka). Els resultats obtinguts també han revelat que aquelles dones que no havien marxat mai de Chukotka tenen concentracions significativament més altes d'HCB, alguns PCBs i mirex que les que han viatjat. No s'han trobat diferències en cap de les dues ciutats argentines segons si les dones són natives o immigrants.

Per tant, en comparar les concentracions de les poblacions, no només és important dividir els voluntaris d'un estudi entre els que viuen en llocs exposats o no exposats (rural/urbà), sino aquells que per dieta o hàbits poden arribar a tenir una exposició diferent (costa/interior).

Influència del nivell educatiu i la classe social en l'acumulació de COPs. Els estudis a l'Argentina i Rússia mostren que el nivell educatiu i la classe social no semblen tenir un paper rellevant en l'acumulació de COPs.

Influència dels OCs en les dones embarassades i els seus nounats. A l'estudi de Rússia (Chukotka) s'ha trobat una associació positiva entre les concentracions de 4,4'-DDT amb l'edat gestacional, el pes i la longitud dels nadons que indica una possible interacció metabòlica entre aquest compost i el fetus. No s'ha trobat cap relació entre el tipus de part, malformacions en el fetus o complicacions durant el part i les concentracions dels compostos organoclorats estudiats. No s'han observat diferències en els models estratificats per sexe perl que no s'observen efectes de feminització.

Influència de la destil·lació global. A l'estudi d'Argentina, s'ha vist que les concentracions dels compostos més volàtils tendeixen a ser més alts a Ushuaia, indret més fred, mentre que els menys volàtils són més alts a Salta. El β-HCH és una excepció a aquesta tendència possiblement per l'ús local a Salta en agricultura i ramaderia. Aquesta diferència de concentracions segons la latitud de les ciutats estudiades pot ser deguda a l'efecte de la destil·lació global.

CAPÍTOL 4. PESTICIDES ORGANOFOSFORATS I PIRETROIDES

Capítol 4: Pesticides organofosforats i piretroides

4.1 Introducció

En els últims 50 anys els compostos persistents que s'utilitzaven en el passat s'han hagut de substituir per altres no persistents.

La gran demanda per part del sector agrícola ha fet que la indústria dels insecticides hagi desenvolupat diferents compostos sintètics o semisintètics amb la finalitat de millorar i potenciar l'activitat insecticida així com combatre resistències que els potencials organismes diana han anat adquirint amb els anys. La cerca de l'insecticida ideal és contínua, i amb el pas del temps s'ha aconseguit un gran ventall de famílies de pesticides, cadascuna amb característiques diferents. Algunes d'aquestes famílies de compostos són els carbamats, els nicotinoides, els piretroides o els organofosforats.

En aquesta memòria de tesi doctoral s'han estudiat alguns dels pesticides organofosforats més utilitzats i els piretroides.

Els pesticides organofosforats

Els pesticides organofosforats tenen estructura general $O=P(OR)_3$, on dos grups –R corresponen a –OMe o –OEt, i l'altre és un grup específic per a cada compost (Figura 4.1). S'utilitzen com a plaguicides artificials per controlar les poblacions d'insectes tant a l'agricultura com en l'àmbit domèstic, en jardins i veterinària. Són pesticides no sistèmics, actuen sobre el sistema nerviós inhibint

l'acetilcolinesterasa, enzim encarregat de la hidròlisi del neurotransmissor acetilcolina, que és una substància implicada en la transmissió dels senyals nerviosos, i provoquen la mort per disfunció neuronal (Tafuri i Roberts, 1987; Mileson et al., 1998). Aquesta inhibició és irreversible, a diferència d'altres insecticides que també actuen de manera similar. A més, no és selectiva, per tant poden afectar també a altres espècies exposades com mascotes o humans.

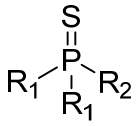

Figura 4.1 Estructura general dels pesticides organofosforats. R_1 correspon a –OMe o –OEt i R_2 és específic de cada pesticida.

Tot i que els primers compostos organofosforats es van sintetitzar el 1851, no va ser fins l'any 1932 que el químic alemany Willy Lange i el seu estudiant Gerda von Krueger van experimentar, per primer cop, els seus efectes neurotòxics en ells mateixos (Petroianu, 2010). Més tard, el 1936, Gerard Schrader va experimentar amb ells com a pesticides. Aviat, es va veure el seu potencial a l'àmbit militar com a gasos neurotòxics. Alguns dels compostos desenvolupats per a aquest ús van ser el gas sarin, tabun o soman (Everts, 2016).

Així als anys 50, després de la Segona Guerra Mundial, es va estendre el seu ús a l'agricultura, sintetitzant-se en grans quantitats. El parató junt amb el malató es van consolidar com uns dels pesticides més populars, el seu ús, a més, es va incrementar degut a la prohibició dels compostos organoclorats.

A continuació es descriuen els pesticides organofosforats estudiats en aquest treball.

El paratió

El paratió (O,O-dietil-O-4-nitro-feniltiofosfat) és un insecticida i acaricida potent, s'aplicava en camps de cotó, plantes d'arròs i arbres fruiters (Figura 4.2). La forma etil, es va registrar per primera vegada als Estats Units l'any 1948. Va ser el segon pesticida organofosforat introduït a l'agricultura i el primer en ser comercialitzat (IARC, 2017). La preocupació envers la salut i el medi ambient va portar al desenvolupament del metilparatió, que és menys tòxic.

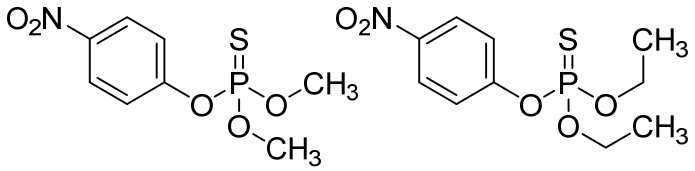

Figura 4.2 Estructures químiques del metil-paratió (esquerra) i de l'etil-paratió (dreta).

En l'actualitat està prohibit en totes les seves formulacions i està classificat per l'Organització Mundial de la Salut com a extremadament perillós (OMS, 2005). El 2004 va ser inclòs a l'annex III del conveni de Rotterdam sobre el procediment de consentiment fonamentat previ aplicable a certs plaguicides i productes químics perillosos objecte de comerç internacional, juntament amb el DDT, HCH, HCB, mirex i PCBs, compostos organoclorats mencionats en el capítol anterior (OMS et al., 2004).

El malatió

El malatió (2-[(dimetoxifosforotioil)sulfonil]butanodioat de dietil) es va introduir l'any 1950 i és un dels pesticides més antics i utilitzats de la família dels organofosforats (Figura 4.3). Té un marge d'actuació molt ampli, s'utilitza en l'agricultura en diferents tipus de cultiu per l'alimentació i farratge, en instal·lacions per guardar el gra, en gespes, jardins, vivers o àrees residencials, en pastures o en programes regionals de eradicació de plagues. A més, a

concentracions baixes (0,5%) s'utilitza en locions capil·lars pel tractament de polls en humans (ATSDR, 2003b).

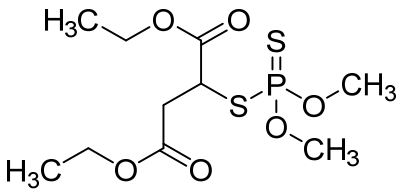

Figura 4.3 Estructura química del malatió.

Encara que l'aprovació del malatió es va revocar el 2008 per la Unió Europea (UE), el 2010 els estats membres de la UE van votar permetre la seva utilització per al control de plagues d'insectes en cultius. Així es va tornar a autoritzar a Àustria, la República Txeca, Grècia, Espanya, Itàlia, Polònia, Romania i Eslovàquia, aquesta autorització esta en procés per Bulgària i Hongria, fins l'any 2022 quan hi haurà una revaloració nova (EC, 2016).

El diazinon

El diazinon (O,O-dietil-O-(2-isopropil-6-metil-pirimidin-4-il)fosforotioat) es va utilitzar com a pesticida per primera vegada a principi dels anys 50 (Figura 4.4). S'utilitzava tant a l'agricultura com en l'àmbit domèstic pel control d'insectes en sòls, plantes ornamentals i cultius de fruita i hortalisses i per controlar plagues casolanes com mosques, puces o paneroles.

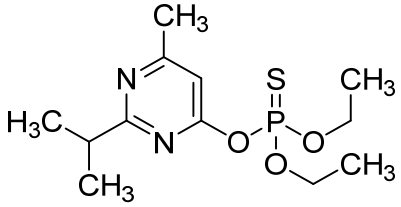

Figura 4.4 Estructura química del diazinon.

A la dècada dels 80 tant els Estats Units com Canadà van suspendre l'ús del diazinon pel control de larves i cucs en camps de golf i granges de gespa degut a les morts de les aus aquàtiques migratòries (ATSDR, 2008). Al final del 2002, als

Estats Units, es va reduir la seva utilització en agricultura en un 30%, i la resta d'usos es van restringir. Totes les aplicacions interiors ja fossin residencials o no, així com els productes per a usos externs en zones residencials o jardins es van anar eliminant gradualment des del 2004 (EPA, 2006).

A Europa, l'any 2006, es va retirar la autorització per a l'ús de productes contenint diazinon sobre camps de cultius o animals (EC, 2016). Actualment, la comercialització i ús del diazinon estan prohibits.

El clorpirifos

El clorpirifos ((O,O-dietil-O-3,5,6-tricloropiridin-2-il)fosforotioat) es va registrar i comercialitzar per primera vegada el 1965 (Figura 4.5). És un pesticida utilitzat àmpliament en habitatges i agricultura pel control de mosques, cotxinilles, erugues o escarabats. No és molt soluble en aigua, de manera que generalment es barreja amb líquids oliosos abans d'aplicar-se.

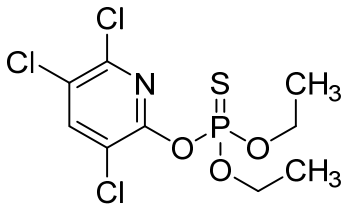

Figura 4.5 Estructura química del clorpirifos.

Un cop alliberat al medi ambient, es volatilitza ràpidament incorporant-se a l'atmosfera. Com s'ha dit abans és poc soluble en aigua, característica que fa que s'uneixi a diferents partícules de plantes i sòls, que l'ajuden a introduir-se als medis aquàtics. Tant a l'aigua com al sòl, és susceptible de sofrir fotodegradació, amb una vida mitjana que pot variar de 6 hores a 3 dies. No obstant això, en ambients interiors pot persistir durant mesos degut a la falta de la llum solar i els microorganismes que contribueixen a la degradació en un ambient extern (Eaton et al., 2008).

El 2007 es va prohibir el seu ús en preparats biocides amb aplicacions ambientals i en la industria alimentària es va limitar el seu ús va a l'agricultura i en jardins a nivell domèstic. Actualment i fins la pròxima reavaluació al gener del 2020 està aprovat el seu ús per la Unió Europea (EC, 2016).

El pirimifos

El pirimifos (Figura 4.6) (O-[2-(dietilamina)-6-metilpirimidin-4-il] O,O-dimetil fosforotioat) és un insecticida desenvolupat l'any 1967, s'utilitza principalment després de la collita per tractar el blat de moro, el sorgo i altres llavors que han de romandre emmagatzemades, s'afegeix a les etiquetes identificadores per bestiar i s'utilitza en tractaments en bulbs d'iris (EPA, 2016). També és un dels insecticides utilitzats pel control dels vector triatomes, un tipus de xinxes implicades en la transmissió de la malaltia de Chagas. En aquest cas, s'afegeix com a additiu a pintures d'ús interior (Rozeendal, 1997).

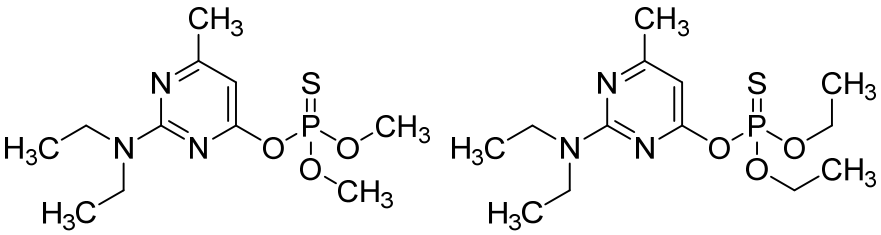

Figura 4.6 Estructures químiques del metil pirimifos (esquerra) i etil pirimifos (dreta).

En l'actualitat la Unió Europea ha autoritzat el metil pirimifos fins a la pròxima revaloració el juliol del 2020 (EC, 2016). L'etil pirimifos no està autoritzat per la Unió Europea encara que aquest insecticida està autoritzat a Bèlgica i Luxemburg.

El coumafos

El coumafos (Figura 4.7) (O,O-dietil-O-3-cloro-4-metil-2-oxo-2H-cromen-7-il fosforotioat) és un insecticida no volàtil, insoluble en aigua. S'utilitza en granges

i en l'àmbit domèstic per tractar els animals de paparres, àcars, mosques i puces. També s'utilitza pel control de Varroa, àcar que es considera una plaga en les colònies d'abelles, arribant-se a trobar residus d'acaricida tant en la cera d'abella, on arriba a persistir fins a 5 anys (Zhu et al., 2014), com en la mel de consum (Karazafiris et al., 2008).

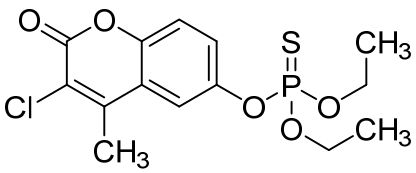

Figura 4.7 Estructura química del coumafos.

Està classificat com a substància extremadament perillosa als Estats Units i actualment no està aprovat per la Unió Europea (EC, 2016).

Els piretroides

Durant els anys 20 es van estudiar les piretrines, que constitueixen un 50% de l'extracte oliós de les flors de piretre (*Chrysanthemum cinerariifolium*). És un insecticida i repel·lent natural que penetra ràpidament en molts insectes paralitzant el seu sistema nerviós. A partir d'aquestes, s'han sintetitzat altres molècules d'estructura similar, els piretroides. S'han utilitzat com a substituts dels pesticides organofosforats, així com aquets havien substituïts els organoclorats (Katsuda, 1999; Amweg et al., 2006; Zhan i Zhang, 2014).

L'avantatge d'aquests insecticides sintètics nous és que posseeixen una activitat insecticida superior, sent més tòxics pels insectes i menys pels mamífers. Són eficients contra un marge ample de plagues de insectes, a més també s'utilitzen com a insecticides domèstics, en camps i hivernacles.

Malhauradament, aquests pesticides són més tòxics en el medi marí (Maund et al., 2012; Bille et al., 2017), i a més, són és resistents a la degradació lumínica, que els fa més persistents en el medi ambient (ATSDR, 2003a; Gajendiran i Abraham, 2018).

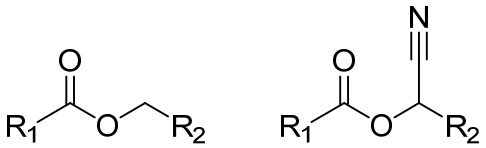

Figura 4.8 Estructura general dels dos tipus de piretroides. Tipus I (esquerra), tipus II (dreta).

Els piretroides es classifiquen en dos grups, tipus I i tipus II, tot depenent de la seva toxicitat i propietats fisicoquímiques. El primer insecticida piretroides utilitzat va ser l'al·letrín que va ser identificat per Milton S. Schechter el 1949 (Bradberry et al., 2005). Pertany al tipus I perquè conté un èster carboxílic del ciclopropà en la posició R_1. El poder insecticida d'aquests compostos es va amplificar amb l'addició d'un grup nitril en la posició α del O donant lloc als piretroides classificats com a tipus II (Figura 4.8) (Gajendiran i Abraham, 2018). D'altra banda es va trobar activitat insecticida similar en uns èsters de 3-fenoxibenzil, que no tenien el ciclopropà, però sí el grup nitril (Bradberry et al., 2005), i es consideren també del tipus II (Figura 4.9).

L'eficàcia, selectivitat, toxicitat i propietats dels piretroides ve determinada per la seva estructura, forma i estereoquímica de l'anell de ciclopropà. Els piretroides de tipus I presenten dos centres estereogènics, per tant, tenen dos diastereòmers i els seus corresponents enantiòmers. Per altra banda els de tipus II tenen tres centres estereogènics que es tradueixen en quatre diastereòmers i les seves quatre imatges especulars (Jin et al., 2012).

Actualment un 25% dels insecticides utilitzats són piretroides (Casida i Quistad, 1998; Shafer et al., 2005). La seva producció total als Estats Units va augmentar de 15 tones/any fins a 630 tones/any del 1945 al 1976 (Ridgway et al., 1978). Tot i que no són persistents ni es bioacumulen (Casida et al., 1975; Leng et al., 1997), el fet de que s'utilitzin mundialment i contínuament fa que es trobin constantment en tot tipus de compartiments ambientals i matrius biològiques (Daughton, 2004).

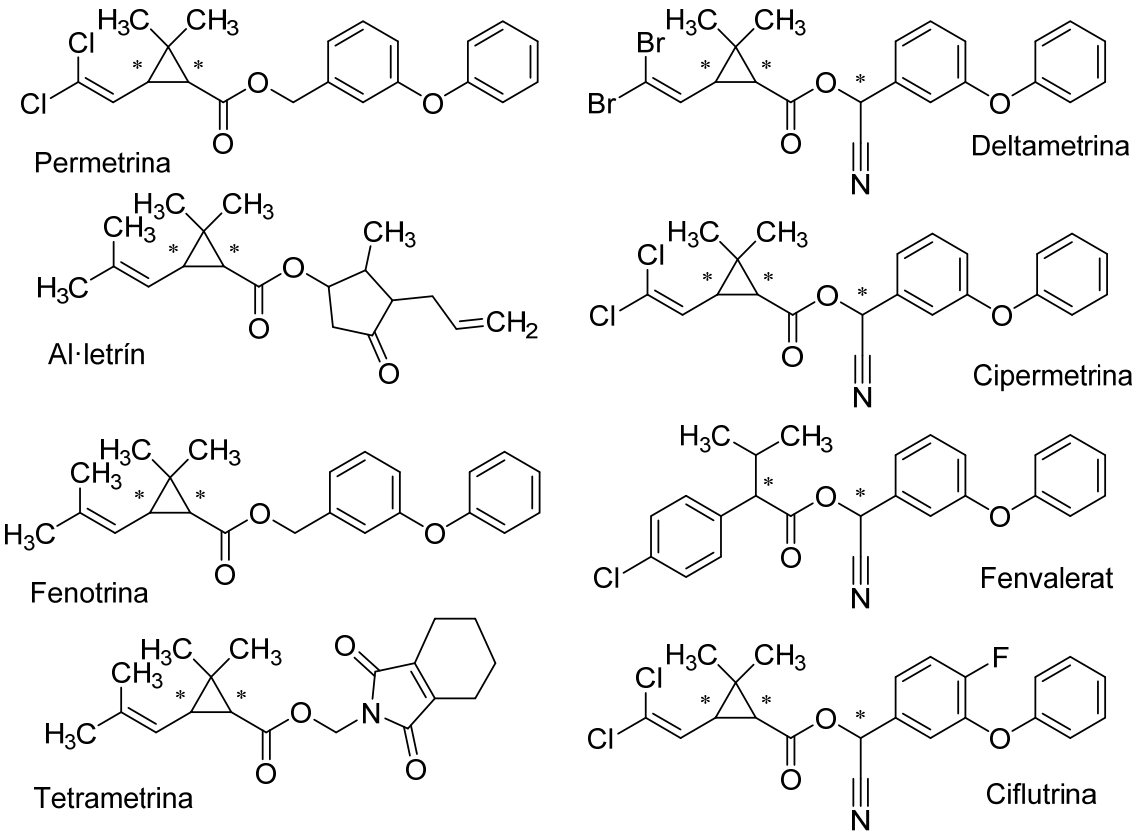

Figura 4.9 Exemples d'alguns dels piretroides més importants del tipus I (esquerra) i tipus II (dreta). Els asteriscs indiquen els centres estereogènics.

Exposició humana a OPs i PYR

Els pesticides s'utilitzen a nivell mundial sobre tot tipus de collites per destruir, prevenir, repel·lir o mitigar plagues d'insectes. Com a conseqüència els humans n'estem inevitablement exposats. Una de les rutes més importants d'exposició entre la població general és, com en el cas dels COPs, a través de la dieta, sobretot en ingerir fruites i verdures (Lu et al., 2008; Holme et al., 2016; Jardim et al., 2018).

Alguns pesticides organofosforats com el malatió o el clorpirifos i alguns piretroides s'utilitzen, a part d'en camps de conreu, a nivell domèstic o en parcs i jardins públics, ja sigui per tractament contra els polls, pel tractament contra plagues en plantes, pel control de puces o àcars en les mascotes o com a

insecticides. D'aquesta manera la població general també esta exposada a través del sistema respiratori com a conseqüència de la seva aplicació en la llar o en zones verdes de les ciutats.

Un dels col·lectius més exposats tant a pesticides organofosforats com a piretroides són els grangers, que són els encarregats de ruixar els camps de conreu per combatre les plagues que hi puguin haver. Aquests estan exposats tant per via respiratòria com per via dèrmica. Per això és important i necessària la utilització d'equips de protecció individual (EPI) adequats per aquesta activitat. L'exposició puntal pot provocar mal de caps, nàusees o irritació (Gari et al., 2018). Altres rutes menors d'exposició es donen per la ingesta d'aigua contaminada o accidentalment per un ús inapropiat (ATSDR, 2003a).

A diferència dels COPs, el nostre cos és capaç de metabolitzar aquests compostos, majoritàriament al fetge, i un cop exposats som capaços d'eliminar els metabòlits ràpidament, principalment a través de l'orina, però també a través de la femta o la respiració (Barr, 2008). Els piretroides tenen una vida mitjana curta al plasma i un elevat volum de distribució als teixits (Fernández A. et al., 2010). Si els nivells d'exposició són molt alts i de manera continuada, es poden emmagatzemar als teixits grassos i romandre al cos un temps més llarg. A més, alguns tipus de piretroides poden quedar retinguts períodes més llargs a la pell o al cabell (ATSDR, 2003a).

Metabolisme dels pesticides OP i els PYR

Com s'ha descrit amb anterioritat, els pesticides organofosforats tenen una gran varietat d'estructures amb la presència comuna d'un àtom de fòsfor pentavalent (Figura 4.1). Les rutes metabòliques que segueixen els pesticides organofosforats estudiats en aquesta tesi són equivalents i es donen en dues fases, Fase I, reaccions d'oxidació, reducció o hidròlisis i Fase II, reacció de conjugació (Chambers et al., 2010).

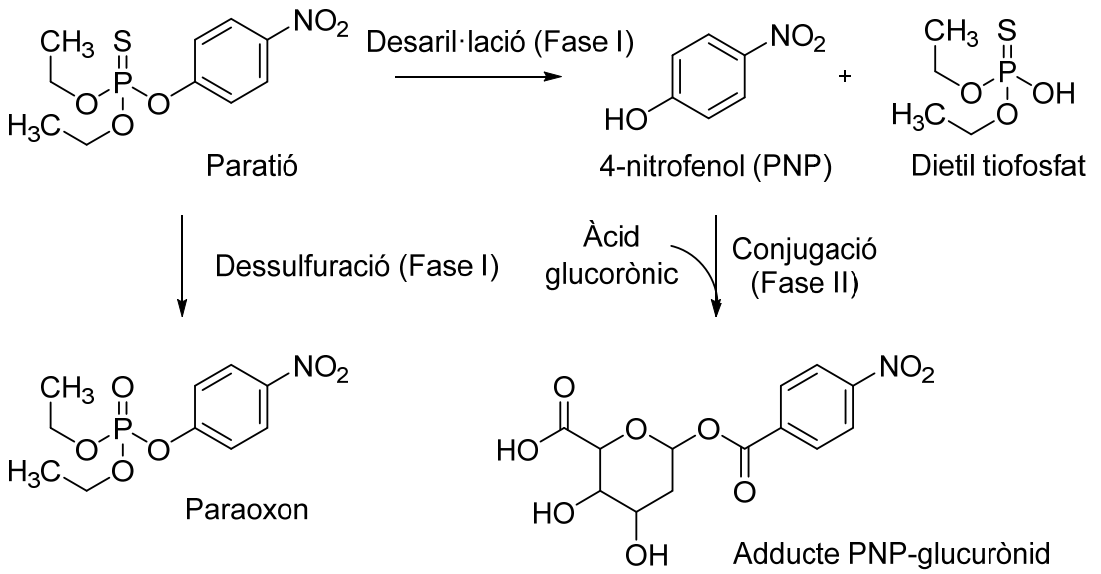

Figura 4.10 Via metabòlica més important en el metabolisme del pesticida organofosforat paratió.

Generalment, durant la Fase I (Figura 4.10) hi ha simultàniament la reacció de dessulfuració i desaril·lació, ambdues catalitzades per l'enzim citocrom P450 (CYP). La dessulfuració és una de les reaccions més importants en el metabolisme dels pesticides OPs, que implica l'atac del fòsfor per H_2O formant un intermedi inestable (fosfooxitiirà) que resulta en la substitució de l'àtom de sofre pel d'oxigen (Chambers et al., 2010), tot formant-se un compost més tòxic, paraoxon en el cas de l'exemple de la Figura 4.10. La reacció de desaril·lació té com intermedi el mateix fosfooxitiirà, amb la diferència que genera el trencament del compost donant lloc a un dialquiltiofosfat i a un grup sortint específic per a cada pesticida, 4-nitrofenol (PNP) en el cas del paratió.

En la Fase II (Figura 4.10) el grup sortint (PNP) es conjuga amb l'àcid glucurònic formant un adducte més soluble en aigua que pot ser excretat en l'orina. Aquesta reacció està catalitzada per la glucuronosil transferasa (Chambers et al., 2010).

No existeix una sola ruta metabòlica per a tots els piretroides, sinó que cadascun segueix una via diferent segons la seva estructura, així doncs, hi ha diferències segons el grup en R_2 (Figura 4.8), si es tracta de l'isòmer *cis* o *trans* o

fins i tot si tenen un alcohol primari o secundari. Una revisió de les vies metabòliques de 30 piretroides diferents van revelar que la seqüència de reaccions metabòliques més comuns són hidròlisi de l'èster, oxidació i conjugació, que es produeixen en dues etapes anomenades Fase I i Fase II, catalitzades per diferents isoformes de l'enzim CYP (Kaneko, 2011).

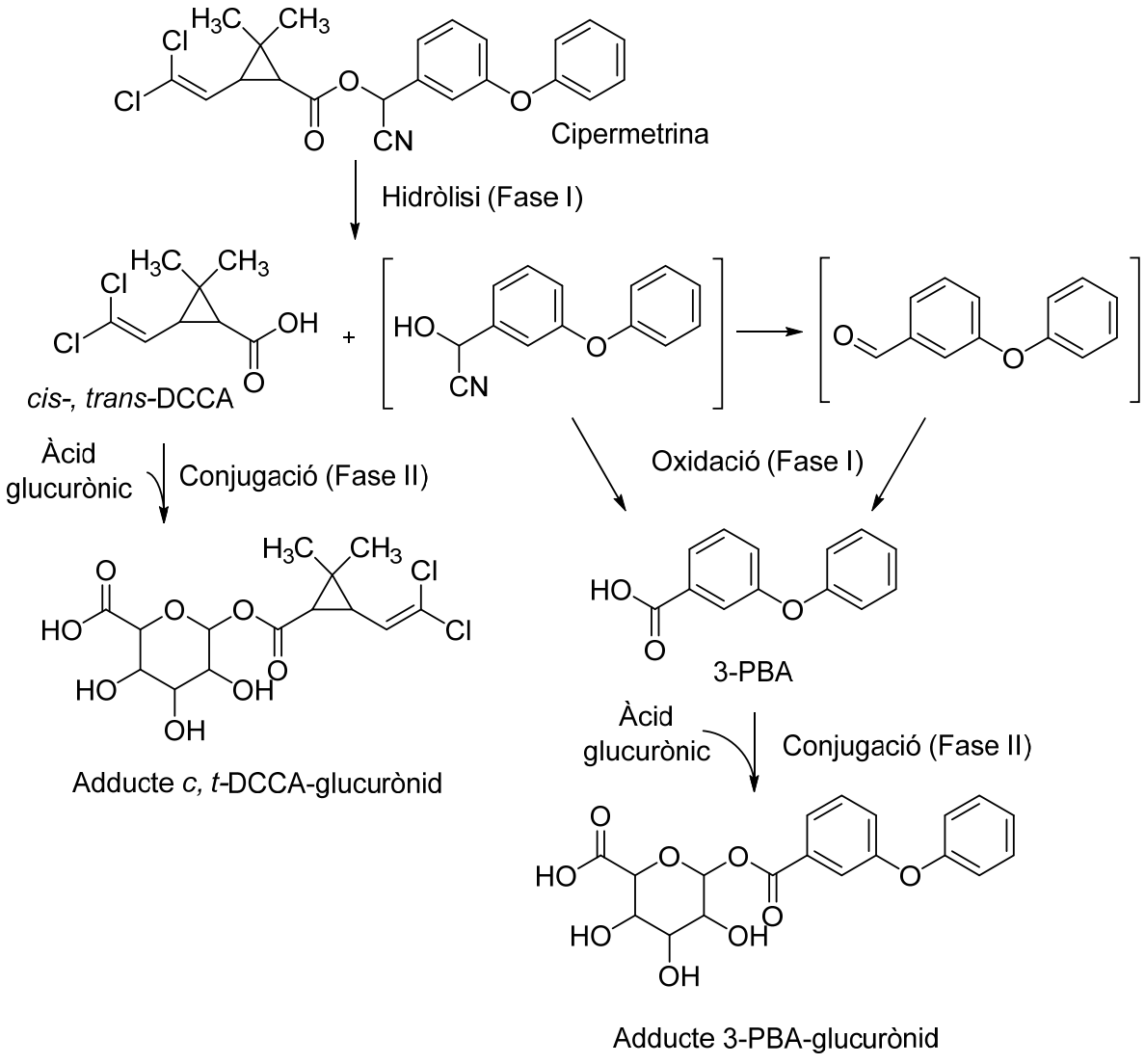

Figura 4.11 Via metabòlica més important en el metabolisme del piretroide cipermetrina.

La hidròlisi de l'èster es produeix durant la Fase I. Aquesta reacció és comú en la majoria de piretroides i dona lloc, entre d'altres, a l'àcid 3-fenoxibenzoic (3-PBA) o l'àcid 4-F-3-fenoxibenzoic (4-F-3-PBA) en el cas de la ciflutrina. En aquesta mateixa fase en alguns dels piretroides es produeixen les reaccions d'oxidació, que tenen lloc en diferents punts del compost depenent de l'estructura química. En la Fase II l'àcid es conjuga, tot formant el metabòlit 3-PBA-6-glucurònid (o 4-F-3-PBA-6-glucurònid), uns dels metabòlits majoritaris en el metabolisme dels piretroides (Figura 4.11). Aquests metabòlits s'excreten ràpida i completament en l'orina i la femta durant pocs dies després de la seva administració oral (Kaneko, 2010).

Com es pot observar a les figures 4.10 i 4.11 cada pesticida té diferents metabòlits en orina. En aquesta tesi s'han analitzat alguns dels més representatius. En el cas de pesticides OPs s'han analitzat els específics de cada un (DEAMPY, IMPY, PNP, CMHC, MDA i TCPY metabòlits de pirimifos, diazinon, paratió, coumafos, malatió i clorpirifos, respectivament) i el 3-PBA i 4-F-3-PBA com a metabòlits genèrics de tot un conjunt de piretroides amb aquests grups en la seva estructura (per exemple, permetrina, fenotrina, deltametrina, fenvalerat o ciflutrina).

Mecanisme d'intoxicació dels pesticides OP i els PYR

En una intoxicació per pesticides organofosforats o per piretroides la reacció del cos a nivell macroscòpic és similar. En tots dos casos sota una intoxicació lleu es poden sentir nàusees, mal de caps o debilitat general. Malgrat això, els mecanismes d'intoxicació són diferents. OPs i PYR actuen sobre les neurones, però en zones diferents, els OPs actuen sobre els terminals sinàptics, mentre que els piretroides tenen un efecte sobre l'axolemma, part de la membrana cel·lular que rodeja l'axó (Figura 4.12).

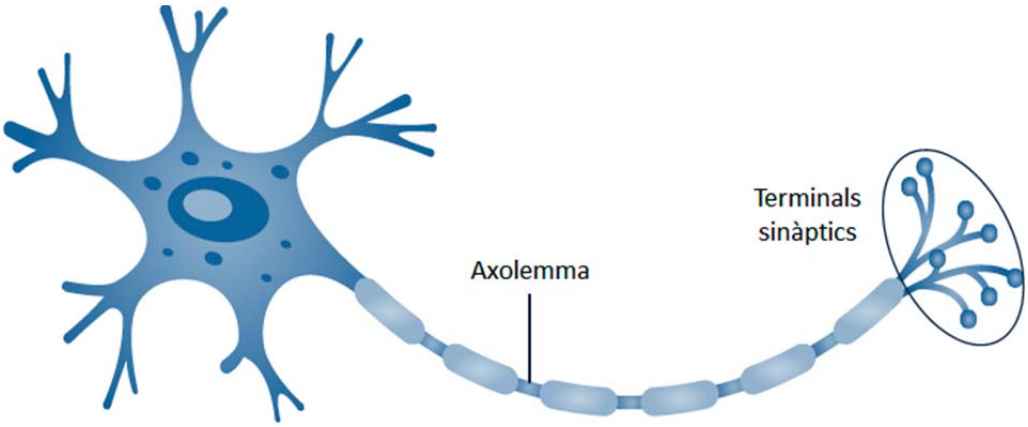

Figura 4.12 Imatge esquemàtica d'una neurona.

En el cas dels compostos organofosforats, aquests desenvolupen la seva toxicitat a través de la fosforilació de l'enzim acetilcolinesterasa en les terminacions nervioses. Reaccionen amb l'ester de l'enzim colinesterasa formant una unió estable i irreversible a no ser que es trenqui mitjançant un tractament específic, quedant l'enzim inutilitzat per a la seva funció normal (Figura 4.13). La pèrdua de la funció enzimàtica permet l'acumulació d'acetilcolina en diferents llocs dels sistema nerviós, tot provocant diferents tipus de reaccions (Eldefrawi i Eldefrawi, 1983).

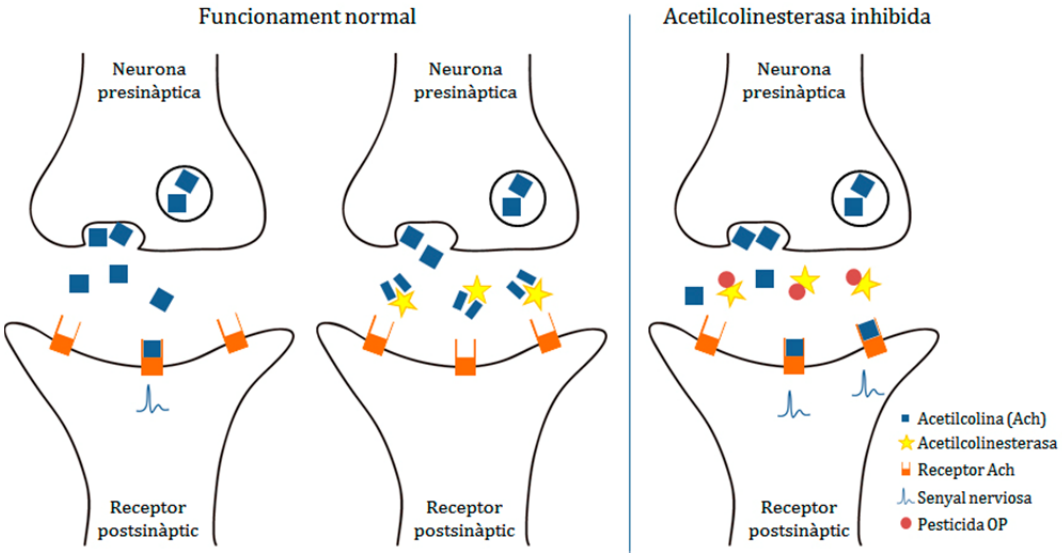

Figura 4.13 Esquema del funcionament normal del neurotransmissor acetilcolina i la seva inhibició per part dels pesticides organofosforats.

L'acetilcolina és un neurotransmissor que interactua amb dos tipus de receptors postsinàptics (nicotínics i muscarínics), i és responsable de la transmissió de l'impuls nerviós. Durant el funcionament normal, un cop alliberada, després d'haver interactuat amb el seu receptor, es destrueix mitjançant l'acció de l'enzim acetilcolinesterasa, on s'hidrolitza i produeix colina i àcid acètic, que entren al pool metabòlic presinàptic per ser utilitzats novament (Fernández A. et al., 2010).

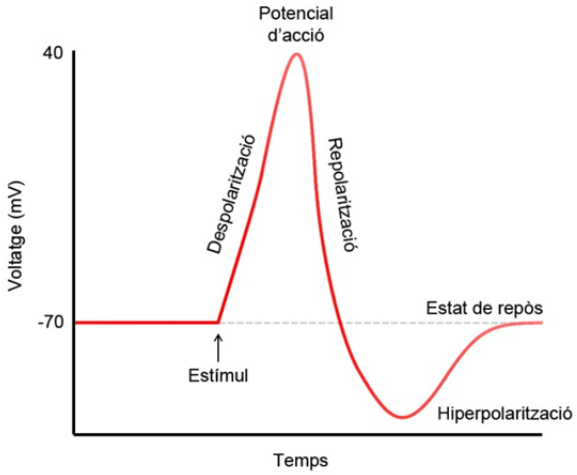

Figura 4.14 Ona de descàrrega elèctrica característica de l'impuls elèctric en la neurona.

El mecanisme d'intoxicació dels piretroides, tot i ser lleugerament diferent entre els de tipus I i II, es caracteritza per produir una inferència reversible en el mecanisme de transport iònic a través de la membra cel·lular de l'axó (axolemma), que interfereix en la funció neuronal i bloqueja els canals de sodi de manera que queden oberts. Això permet permet l'entrada d'ions sodi sense regulació. Com a conseqüència, els nervis no es poden repolaritzar i causa la paralització de l'organisme (Figura 4.14). Quan es funciona de forma normal, els canals de sodi s'obren i tanquen per regular el potencial a fora i a dins de les neurones (Figura 4.15).

La diferència més significativa entre els dos mecanismes d'acció és que la intoxicació per pesticides organofosforats és irreversible, mentre que en el cas

dels piretroides és reversible. Aquesta diferència fa que els piretroides siguin menys tòxics per humans i grans mamífers que els pesticides organofosforats, un dels motius pels que en l'actualitat s'estan utilitzant més piretroides que pesticides organofosforats.

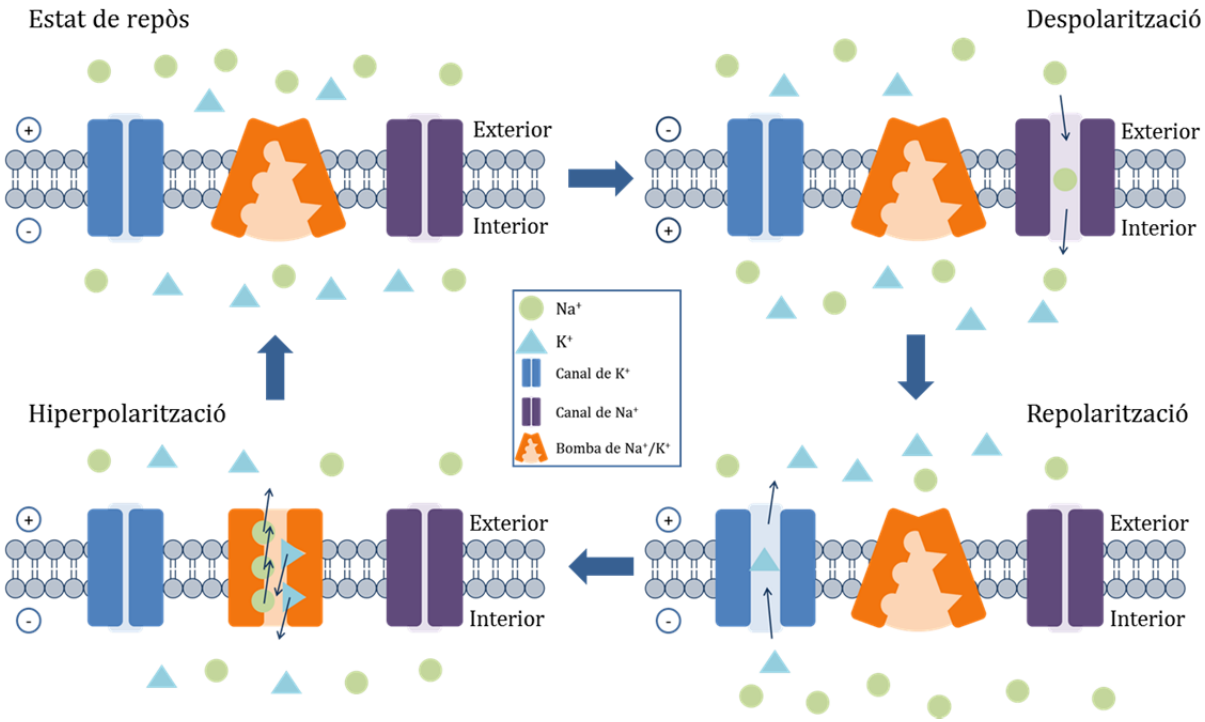

Figura 4.15 Esquema del funcionament normal del impuls elèctric que viatge al llarg de la membrana de una neurona.

Poblacions d'estudi

Respecte a pesticides organofosforats i piretroides s'han estudiat diferents poblacions de tres països europeus, tots ells membres de la Unió Europea: Itàlia, Eslovènia i Espanya. A Itàlia s'ha estudiat una població de nens de la ciutat de Trieste, a Eslovènia parelles de mares i nens de Ljubljana i a Espanya una població de dones embarassades de Tarragona i dues més de població general presumptament exposada i no exposada de Catalunya (Sucs) i Galícia (Carbia i Santiago de Compostel·la).

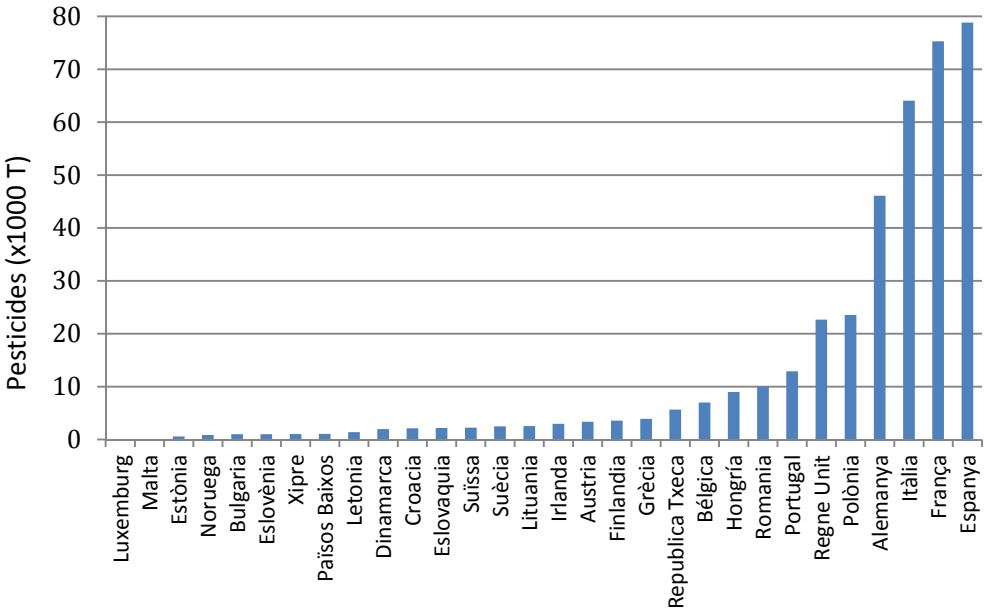

Figura 4.16 Relació de les compres de pesticides a països d'Europa durant l'any 2014.

Segons les estadístiques d'Eurostat, Itàlia i Espanya són uns dels països europeus que compren més pesticides juntament amb França i Alemanya. Per la seva banda, Eslovènia és un dels països on és consumeixen menys (Figura 4.16). La idea inicial a l'hora d'analitzar aquestes mostres recau en poder comparar, en primer lloc, les concentracions de pesticides OPs i PYR en diversos països europeus que tenen un consum diferent de pesticides. En segon lloc, determinar si el fet de viure en una regió rural o urbana influeix en l'exposició a pesticides i per últim identificar alguns dels factors que poden determinar una major exposició als diferents tipus de pesticides.

A continuació es detallen alguns dels aspectes més rellevants dels països i ciutats estudiats (Figura 4.17).

Itàlia - Trieste

Itàlia s'ubica al centre del Mar Mediterrani, el nord esta envoltat pels Alps i té frontera amb Suïssa, França, Àustria i Eslovènia. Té una superfície de 303.340 km^2 i el 2016 hi vivien uns 60,5 milions d'habitants.

Figura 4.17 Localització geogràfica de les poblacions estudiades a Europa.

El turisme és un dels sectors econòmics que ha crescut més en els últims anys, sent un dels països amb més turisme del món. L'activitat industrial ha estat el motor de desenvolupament italià i l'actual eix de la seva economia. Les activitats agrícoles a Itàlia han experimentat un retrocés considerable respecte a anys anteriors, és extensa en cultius de cereals, lleguminoses, plantes industrials, hortalisses, fruita i flors. A més, és un dels majors productors de vi i oli.

Trieste és la capital de la regió de Friuli-Venecia Julia al nord-est d'Itàlia, esta situada al Golf de Trieste, entre la part més septentrional del Mar Adriàtic i la zona càrstica del Carso. Es caracteritza per tenir un àrea industrial i un port importants. La major part de la contaminació ambiental que es troba a Trieste prové d'una fosa de ferro i una incineradora localitzats a la zona industrial de la ciutat (Giglio et al., 2017).

En referència a l'agricultura, aquesta no representa una activitat econòmica important en cap de les sis municipalitats de la província de Trieste. Encara que

la seva morfologia a base de terrasses garanteix unes condicions molt bones pel cultiu de vinyes i oliveres, aquest ha anat disminuint en els últims anys (Mauro, 2011).

Eslovènia - Ljubljana

Eslovènia limita amb Itàlia, el mar Adriàtic, Croàcia, Hongria i Àustria. És el punt de trobada de quatre accidents geogràfics europeus importants: els Alps, els Alps Julians, la plana de Pannònia i la regió del càrstica de Trieste, a més del Mar Mediterrani. Amb una superfície de 20.675 km^2 és un dels països més petits i té una població d'uns 2 milions d'habitants. Només el 6% de la població d'Eslovènia es dedica a la industria primaria tradicional, agricultura, silvicultura i pesca.

Llubljana és la capital i ciutat més gran d'Eslovènia. La industria és el sector econòmic més important de la ciutat, sobre tot en l'àmbit farmacèutic, petroquímic i alimentari. Altres àmbits també importants són la banca, les finances, el transport, la construcció, el comerç i el turisme. Tenint en compte que Ljubljana no és una regió agrícola i a Eslovènia la dedicació a l'agricultura és molt petita, l'exposició més probable a pesticides per als habitants de Ljubljana és mitjançant la dieta o pel seu ús privat en les llars.

Espanya - Tarragona, Sucs, Carbia i Santiago de Compostel·la

Espanya, amb una superfície de 505.990 km^2, és un dels estats més extensos de l'Europa occidental i un dels més poblats d'Europa (46,5 milions d'habitants). Limita amb França i el Principat d'Andorra al nord, Portugal a l'oest, està envoltada del Mar Cantàbric i l'Oceà Atlàntic pel nord i la Mar Mediterrània al sud.

Tradicionalment Espanya ha estat un país agrícola i encara ara és un dels majors productors de l'Europa occidental, encara que des de mitjans dels anys 50 el creixement de la industria va ser molt ràpid, arribant a tenir un pes molt

gran en la seva economia. Al llarg del territori i segons les condicions climàtiques es produeixen diferents tipus de cultius, sent els més importants els cereals, l'arròs, patates i hortalisses, arbres fruiters, vinyes i oliveres.

De les quatre poblacions estudiades dues d'elles són de Catalunya (Sucs i Tarragona) i les altres dues de Galícia (Carbia i Santiago de Compostel·la).

Catalunya, té una extensió de 32.000 km^2 i el 2018 hi vivien uns 7,5 milions d'habitants. Aproximadament la meitat de la població viu a l'àrea metropolitana de Barcelona, mentre que la densitat de població en zones rurals és molt escassa. La posició estratègica de Catalunya al Mar Mediterrani li ha permès establir-se com a porta comercial amb el sud d'Europa assegurant-se un gran desenvolupament a nivell industrial. A més, també hi ha una activitat notable en el sector agrícola, ramader i, encara que menys, també pesca.

Sucs és una entitat municipal descentralitzada de Lleida (pedania). El municipi va estar despoblat des del segle XVII fins a las segona meitat del segle XX, quan l'Institut Nacional de Colonització el va recuperar amb la intenció de crear un municipi agrícola i ramader (Sucs, 2018). A diferència de Sucs, Tarragona és una ciutat costanera gran. Part de la seva economia recau en el comerç marítim de càrregues grans, petroli, cereals i carbó. El sector industrial ocupa a gran part de la població activa, el complex petroquímic de Tarragona és el més important d'Espanya, a més en els municipis veïns es poden trobar altres indústries com Repsol, Bayer o BASF. Tot i que la industria a Tarragona és molt important, la zona d'estudi està ubicada en una àrea de producció d'oli d'oliva, vi i avellanes.

Galícia, situada al nord-oest de la península ibèrica, té una superfície de 29.575 km^2 i una població de 2,7 milions d'habitants. L'economia de Galícia està fortament enllaçada als recursos naturals. Tradicionalment les activitats del sector primari han estat les predominants, encara que amb els anys han anat disminuint en pes.

Santiago de Compostel·la és una ciutat urbana de la província de La Corunya. L'economia de la ciutat depèn bàsicament del sector terciari, tenint un 12% de treballadors dedicats a la industria o la construcció, només un 1% dels habitants en actiu es dediquen a l'agricultura (IGE, 2017). La ciutat de Carbia és un municipi rural de la província de Pontevedra que està a uns 40 km de Santiago de Compostel·la. La població d'aquesta regió es va incloure a l'estudi per poder comparar una ciutat urbana com Santiago de Compostel·la amb una de rural com Carbia.

4.2 Metodologia

Disseny dels estudis

Les poblacions d'Itàlia i la d'Eslovènia formen part del projecte PHIME (*Public Health impact of long-term, low-level mixed element exposure in susceptible population strata*), que es va dissenyar per estudiar exposicions ambientals i efectes neuropsicològics en nens (Valent et al., 2013).

La població d'estudi de Tarragona consta d'una cohort de dones embarassades i una cohort de naixement en curs (EXHES) que forma part del projecte Europeu HEALS (*Health and Environment-wide Associations based on Large population Surveys*), que té com a objectiu general el perfeccionament d'una metodologia integrada i l'aplicació de les eines analítiques i computacionals per la caracterització de l'exposoma i la realització d'estudis d'associació entre contaminants ambientals i efectes en la salut (HEALS, 2013).

Les mostres de les diferents poblacions de Galícia i de Sucs a Catalunya es van obtenir gràcies a la participació voluntària d'habitants d'aquestes regions. Carbia i Santiago de Compostel·la representen l'exposició rural i urbana, respectivament, mentre que a Sucs es va demanar la participació tant de

població que es dediqués a l'agricultura com de població que no s'hi dediqués però que visqués en el poble per determinar així l'exposició laboral.

Anàlisi de metabòlits de pesticides OP i PYR

En la present memòria de tesi doctoral s'ha desenvolupat un mètode per a l'anàlisi de metabòlits de pesticides organofosforats i piretroides en orina. El mètode i la seva validació es descriuen a l'ARTICLE 4 de la secció de Resultats (Capítol 4). Els aspectes referents a la neteja del material són equivalents als descrits per a l'anàlisi de compostos organoclorats i organobromats (Capítol 3, secció Determinació d'OCs i PBDEs). A continuació s'especifiquen alguns punts de la metodologia que no apareixen a l'article o no s'han considerat anteriorment.

Control de qualitat de les anàlisis

Per assegurar la validesa del procediment analític, així com l'exactitud i precisió durant el desenvolupament del mètode i els estudis posteriors s'han tingut en compte alguns paràmetres: blancs de procediment, punts de qualitat de control i participació a un programa d'intercalibratge internacional.

Com s'ha descrit a l'ARTICLE 4, per minimitzar l'efecte matriu de l'orina els blancs de procediment, els punts de control de qualitat i les rectes de calibratge es van realitzar en orina sintètica.

Programa d'intercalibratge G-EQUAS

El mètode analític utilitzat es va validar per a l'anàlisi de materials de referència proporcionats gràcies a la participació en el programa d'intercalibratge G-EQUAS (G-EQUAS, 2018).

Des del 1982, G-EQUAS (*The German External Quality Assessment Scheme – For Analyses in Biological Materials*) realitza periòdicament un programa de

garantia de qualitat i certificació per anàlisis de materials biològics (sang, plasma/sèrum i orina) en l'àmbit de la toxicologia ambiental i laboral. El programa, l'avaluació i la certificació es desenvolupen d'acord amb les directrius de l'Associació Mèdica Alemana (Bundesärztekammer).

Aquest programa està obert a tots aquells laboratoris interessats, i el nostre laboratori hi participa des del 2016, moment en el que es va començar el desenvolupament del mètode per a pesticides OPs i PYR. Dues vegades l'any els participants rebem les mostres a analitzar, que s'analitzen utilitzant el mateix procediment que s'empra per a analitzar mostres reals.

El mètode analític per a la determinació de pesticides OPs i PYR en mostres d'orina es realitza satisfactòriament sota els estàndards de G-EQUAS.

Anàlisi de dades

L'anàlisi de dades així com els gràfics s'han realitzat amb el programa estadístic R (R, 2018).

Les anàlisis inclouen estadística descriptiva i inferència (regressions lineals i anàlisis no paramètriques), així com estimacions d'alguns paràmetres d'interès com l'estimació de la ingesta diària de pesticides. A la secció de Resultats s'indiquen en detall cadascuna de les anàlisis realitzades.

Ajust dels resultats

La manera més senzilla d'expressar els resultats analítics d'OPs i PYR és utilitzar massa per unitat de volum (per exemple ng/mL). Malgrat això, la concentració dels materials dissolts en orina canvia al llarg del dia. Entre altres aspectes, segons la quantitat d'aigua que es beu. Aquests canvis no estan relacionats amb l'exposició. Per això, cal ajustar les dades per minimitzar la influència d'aquests factors que en poden confondre la interpretació de l'exposició.

Existeixen diversos mètodes per ajustar les dades d'orina, tots ells amb els seus avantatges i inconvenients. El més utilitzat és l'ajust per creatinina, un compost de degradació de la creatina que es metabolitza al fetge i s'excreta a l'orina. En condicions normals les persones tenen uns intervals dins d'uns valors. Malgrat això, si la persona té alguna malaltia renal esl nivells poden canviar molt. També influeixen en els nivells de creatinina factors com l'edat, el sexe, la salut renal o fins i tot l'hora del dia en que s'ha pres la mostra (Boeniger et al., 1993; Barr et al., 2005; Suwazono et al., 2005). Per aquest motiu un altre mètode que s'està utilitzant recentment és l'ajust per la densitat de l'orina, que permet tenir en compte la diferent dilució de les diferents orines segons l'aigua beguda.

En els estudis realitzats en aquesta tesi s'han ajustat els resultats per creatinina i densitat sempre que ha estat possible. L'ajust per creatinina s'obté dividint el resultat no ajustat (ng/mL) pel valor de creatinina i el resultat final s'expressa en micrograms d'anàlit per gram de creatinina (μg/g creatinina). Per obtenir la correcció per la densitat es multiplica el resultat sense ajustar (ng/mL) per la relació $(\bar{x}_{SG}-1)/(SG-1)$, on SG, sigles en anglés de *specific gravity,* representa la densitat de cada orina i $\bar{x}_{SG}$ és la mitjana de totes les densitats. El resultat final s'expressa en nanogram d'anàlit per mil·lilitre d'orina ajustats per la gravitat específica (ng/mLajustat per SG).

4.3 Resultats

El capítol 4 està dedicat a estudiar alguns dels pesticides no persistents més importants i utilitzats en les últimes dècades, pesticides OPs i piretroides. S'ha desenvolupat un mètode per a l'anàlisi en orina i s'han avaluat diferents poblacions Europees, quatre d'elles, Trieste, Tarragona, Sucs i Galícia, en països on el consum de pesticides és dels més elevats d'Europa i una altra a Ljubljana on es consumeixen menys pesticides. Els resultats es componen dels següents estudis:

- Desenvolupament d'un mètode per a l'anàlisi dels metabòlits de pesticides organofosforats i piretroides en orina (Article 4 – publicat a la revista *Science of the Total Environment*, 2018).
- Estudi de la càrrega de pesticides organofosforats i piretroides en una població de nens de 7 anys de Trieste (Itàlia) (Article 5 – publicat a la revista *Environmental Research*, 2019)
- Determinació dels nivells de pesticides organofosforats i piretroides en parelles de mares i fills de Ljubljana (Eslovènia) i comparació entre ells (Article 6 – enviat a *Environmental International*)
- Anàlisi de la variabilitat en orina de pesticides organofosforats en dones embarassades del Camp de Tarragona (Article 7 – enviat a *Environmental Research*)

ARTICLE 4

Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural popoulations (Catalonia and Galicia).

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Science of The Total Environment 622-623, 526-533 (2018)

DOI 10.1016/j.scitotenv.2017.11.355

Science of the Total Environment 622–623 (2018) 526–533

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia)

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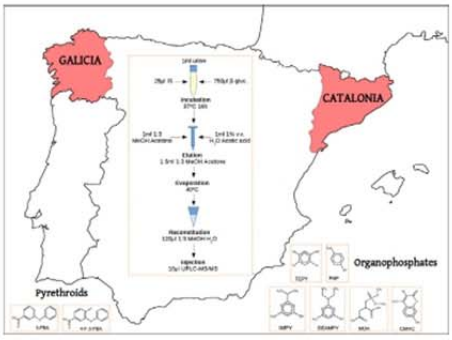
HIGHLIGHTS

- An UPLC-MS/MS method for analysis of urine organophosphate metabolites was developed.
- An UPLC-MS/MS method for analysis of human urine pyrethroid metabolites was developed.
- The use of synthetic urine afforded calibration straight lines with lower detection limits.
- Detection limits were in the range of 14–69 pg/ml.
- Organophosphate concentrations in farmworkers was twofold than in urban populations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 September 2017
Received in revised form 29 November 2017
Accepted 30 November 2017
Available online 13 December 2017

Keywords:
Ultra-performance liquid chromatography
Tandem mass spectrometry
Urinary metabolites
Organophosphate pesticides
Pyrethroids

ABSTRACT

Isotope dilution solid phase extraction UPLC-MS/MS has been used to develop a robust and rapid methodology for the determination of eight specific metabolites of organophosphate and pyrethroid pesticides in human urine. The use of methanol:acetone (25:75 *v*/v) affords an improvement in extraction efficiency in comparison to these individual solvents. The use of synthetic urine improves selectivity and limits of detection for the calibration straight lines. The method provides detection limits of 14–69 pg/ml and 18–19 pg/ml for the organophosphate and pyrethroid metabolites, respectively. Urine analyses of these metabolites in urban non-occupationally exposed individuals and farm workers shows that ingestion of these pesticides occurred in both populations. The concentrations of organophosphate pesticide metabolites in the latter were twofold than those from non-exposed populations.

1. Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture as well as for domestic and gardening use. They eliminate insects because of their strong potential to disrupt the brain and nervous system of these organisms. Unfortunately, this neurotoxic effect is not selective enough as to avoid damage to other non-target species, including humans (Barr, 2008). There is growing public concern on pesticide use not only for the negative impacts on wildlife and the environment but also for the potential adverse health effects on humans. OP and PYR pesticide exposure has been related to several

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https://doi.org/10.1016/j.scitotenv.2017.11.355
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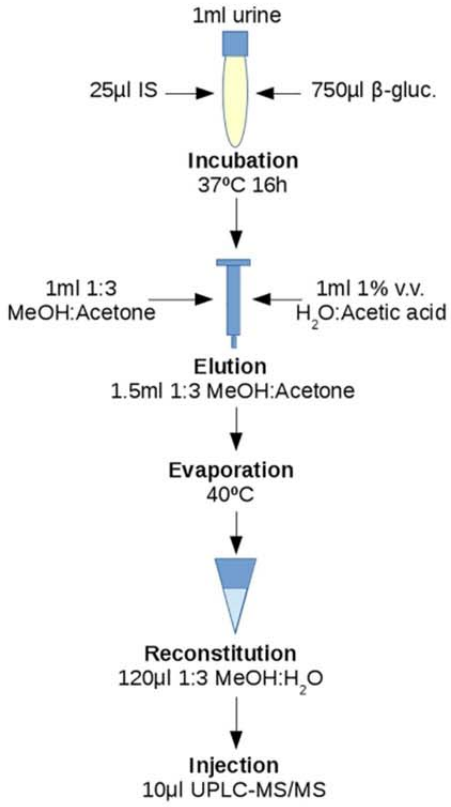

Fig. 1. Graphical representation of the extraction procedure.

health effects, including respiratory, digestive, reproductive and neurological problems, among others (Ye et al., 2013; Arcury et al., 2016; Llop et al., 2017).

Once in the human body, OP and PYR pesticides are typically metabolized and excreted in urine within 4–48 h after exposure, depending on the compound (Egeghy et al., 2011). Organophosphates are metabolized into dialkyl phosphates (DAPs) and specific compounds, including 3,5,6-trichloro-2-pyridinol (TCPY, the metabolite of chlorpyriphos), 4-nitrophenol (PNP, metabolite of parathion), malathion dicarboxylic acid (MDA, metabolite of malathion), 3-chloro-4-methyl-7-hydroxycoumarin (CMHC, metabolite of coumaphos), 2-isopropyl-6-methyl-4-pyrimidiol (IMPY, metabolite of diazinon) and 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY, metabolite of pirimiphos). For the most common pyrethroids, which include permethrin, cypermethrin, deltamethrin and esfenvalerate, all these pesticides are metabolized into one single compound, 3-phenoxybenzoic acid (3-PBA). Cyfluthrin pesticide is metabolized into 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Therefore, 3-PBA and 4-F-3-PBA can be used as a biomarker of the most common PYR pesticides (Barr, 2008; Ueyama et al., 2010; Egeghy et al., 2011).

Urine analysis is the simplest and least intrusive method for assessing human exposure to the aforementioned non-persistent pesticides. Previously published methods for the analysis of specific metabolites of OP and PYR pesticides in urine are based on both gas and liquid chromatography, and mainly using mass spectrometry techniques (Koureas et al., 2012). The concentrations of metabolites of these compounds in urine reflect the exposure levels of the individuals (Barr, 2008). Farmworkers and rural populations are in principle potentially more exposed to these pesticides than general populations (Arcury et al., 2007). However, the low concentrations of these metabolites in urine, currently in the order of ng/ml, and the large numbers of samples needed for epidemiological studies require robust, cheap and efficient analytical methodologies (Barr, 2008). In this context, the limits of detection (LD) are critical to discriminate for the presence of the analytes and for feasibility of study of high numbers of individuals and possible health effects (Currie, 1997; Koch et al., 2001; Ye et al., 2013).

In many of these epidemiological or population toxicity studies these limits are not only considered as analytical parameters but as reference for classification between individuals (Ueyama et al., 2010; Davis et al., 2013; Olsson et al., 2004; Barr et al., 2010; Koureas et al., 2012; Roca et al., 2014a, 2014b). Fulfilling the requirements for the use of detection limits following this approach requires extraction procedures adapted to the most representative conditions of real samples (Garí and Grimalt, 2010). In this context, interferences from human urine may increase limits of detection and distort calibration straight lines. Thus, the developed methodology must consider matrix effects and their variability. The use of synthetic urine instead of urine dilution may provide robust procedures to fulfill these requirements.

Accordingly, a new analytical methodology for the quantification of OP and PYR urinary specific metabolites has been developed in the present study. This method takes into account the variability of concentrations found in human urines from both general and highly exposed populations from rural or agricultural sites. The method is based on ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) and allows the quantification of eight biomarkers of several of these pesticides using only one ml of urine. It provides high precision and accuracy, and low detection limits to analyze these pesticide metabolites both in professionally exposed farmers and non-exposed general population.

2. Materials and methods

2.1. Standards, solvents and reagents

Standards of IMPY and TCPY were purchased from Sigma-Aldrich (Madrid, Spain), PNP from Supelco (Madrid, Spain), CMHC from Acros Organics (Geel, Belgium), DEAMPY, MDA, 3-PBA and 4-F-3-PBA from Dr. Ehrenstoffer (Augsburg, Germany). The isotopically-labeled

Table 1
Instrumental analytical data of the organophosphate and pyrethroid pesticide metabolites considered in the present study.

Acronym	Analyte	Q-SRM[a]	C-SRM[b]	Ion ratio	Collision energy	Cone voltage	Retention time
DEAMPY[c]	2-diethylamino-6-methyl pyrimidin-4-ol	182–154	182–84	1.3	20	40	4.65
IMPY[c]	2-isopropyl-6-methyl-4-pyrimidiol	153–84	153–70	1.9	20	40	5.05
MDA[d]	Malathion dicarboxylic acid	273–141	273–157	2.6	8	25	8.65
PNP[d]	4-nitrophenol	138–108	138–92	8.2	20	45	8.66
CMHC[d]	3-chloro-4-methyl-7-hydroxicoumarin	209–145	209–117	3.2	25	20	9.70
TCPY[d]	3,5,6-trichloro-2-pyridinol	196–196	198–198	1.0	7	10	11.28
3-PBA[d]	3-phenoxybenzoic acid	213–93	213–169	1.6	20	30	12.87
4-F-3-PBA[d]	4-fluoro-3-phenoxybenzoic acid	231–187	231–93	1.3	15	25	13.04

[a] Q-SRM: Quantification Selected Reaction Monitoring.
[b] C-SRM: Confirmation Selected Reaction Monitoring.
[c] Positive ion mode.
[d] Negative ion mode.

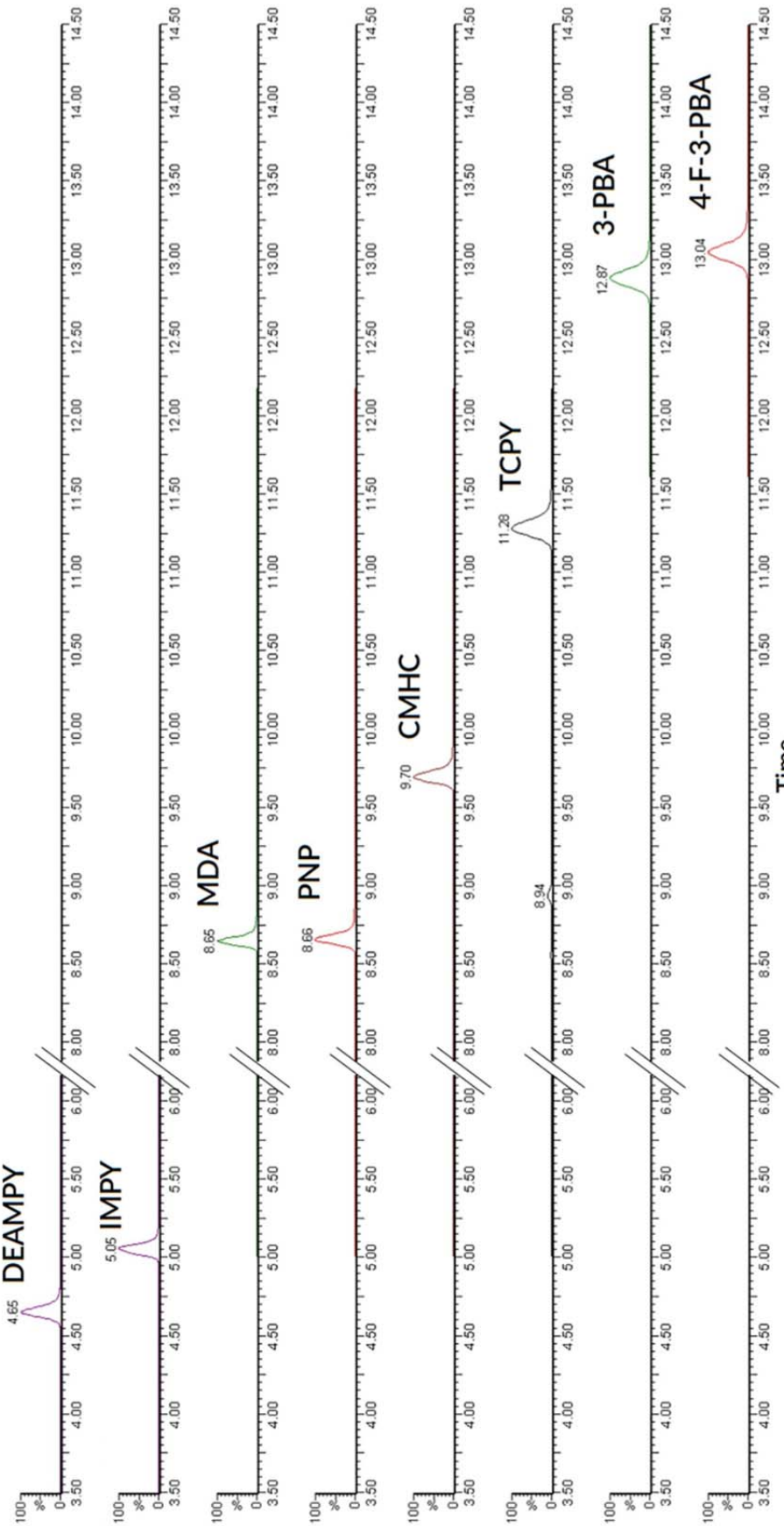

Fig. 2. Chromatogram of a synthetic urine extract showing the peaks of the analytes for selected ion transitions. The x-axis shows the eluting time. Acronyms in Table 1.

Table 2
Limits of detection, recoveries, repeatability and reproducibility of the analyses of the organophosphate and pyrethroid pesticide metabolites.

	LD (ng/ml)	Recovery (%)		Repeatability[a]		Reproducibility[a]	
		QCL	QCH	QCL	QCH	QCL	QCH
DEAMPY[b]	0.017	76	93	4.0	6.9	6.3	5.7
IMPY	0.014	74	97	6.7	6.0	11	11
MDA	0.069	74	73	4.7	6.6	16	17
PNP	0.017	95	87	5.7	2.9	11	8.5
CMHC	0.026	66	74	9.0	5.3	11	11
TCPY	0.020	70	78	5.3	4.4	11	11
3-PBA	0.018	94	84	3.1	2.3	9.7	5.5
4-F-3-PBA	0.019	100	96	3.0	2.3	6.9	6.5

[a] Coefficients of variation (%).
[b] Acronyms in Table 1.

standards were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents used were of analytical grade. Acetonitrile was from Panreac (Barcelona, Spain), methanol, acetone and water for HPLC were from Merck (Darmstadt, Germany), glacial acetic acid was from Scharlab (Barcelona, Catalonia, Spain), and sodium acetate anhydrous and β-glucuronidase type H-1 from *Helix pomatia* were from Sigma-Aldrich (Madrid, Spain).

2.2. Extraction procedure

The method developed here was based on previously reported procedures for the extraction of urinary insecticide metabolites but with substantial modification (Davis et al., 2013; Olsson et al., 2004). Prior to analysis, the urine samples were centrifuged and filtered. Then, one ml was introduced into 10 ml centrifuge tubes. 25 μl of a mixture of the available isotopically labeled internal standards was added. To hydrolyze possible glucuronide or sulfate conjugated metabolites, β-glucuronidase type H-1 from *Helix pomatia* with a specific activity of ~500 units/mg, was used. For a 10-batch sample, 7.50 ml of a buffer solution containing 33.3 mg of β-glucuronidase was used, giving a minimum of 990 units of activity per sample. The samples were incubated overnight at 37 °C and then extracted using solid-phase extraction (SPE). SPE cartridges (Oasis HLB 3 cm^3, Waters, Milford, MA, USA) were preconditioned with 1 ml of methanol/acetone (25:75 *v*/v) followed by 1 ml of HPLC H_2O containing 1% acetic acid. The sample was added and passed through the cartridge. Then the cartridges were washed with 500 μl of HPLC H_2O containing 1% acetic acid and dried for 20 min using vacuum. A solution containing methanol:acetone (25:75 *v*/v, 1.5 ml) was used for eluting the cartridge. The collected extracts were reduced to near dryness under a stream of pure nitrogen. Then, they were quantitatively transferred to chromatographic vials using 120 μl of methanol:water (25:75 *v*/v). An schematic view of the extraction procedure is shown in Fig. 1.

2.3. Instrumental analysis

Compound analysis was performed using Ultra-Performance Liquid Chromatography (UPLC Acquity H-class, Waters, Milford, MA, USA) coupled to a Triple Quadrupole Mass Spectrometer (XEVO-TQ-S, Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. The chromatographic separation was performed on a Betasil C18 column (100 mm × 2.1 mm, 3 μm particle size, Thermo Scientific, West Palm Beach, FL, USA). To extend the life of the column, one guard holder (2.1 μm and 3.0 mm id, Universal Uniguard Holder, Thermo Scientific, West Palm Beach, FL, USA) and a guard column of the same sorbent material (Thermo Scientific, West Palm Beach, FL, USA) were installed inline before the column.

The injection volume was 10 μl, at a flow rate of 0.3 ml/min. The column temperature was kept at 30 °C during the analysis. A gradient elution with a mobile-phase of acetonitrile and a mixture of HPLC H_2O with 1% acetic acid and 5% methanol was used for analysis. The gradient started with ACN/Mixture 2:98, increased to 20:80 in 4 min, then to 40:60 in another 3 min, to 50:50 at minute 14, and finished with 100% ACN at minute 16.5. During the following 3 min the column was cleaned with 100% ACN, adjusted to the initial conditions in minute 19.5, and finally equilibrated for an additional 2.5 min.

Total run time was 22 min. During this interval the MS acquisition parameters changed following three distinct timed segments. In the first, data were acquired in positive ionization mode, and the total run time was 5 min (from minute 3 to 7). In the second and third, data were acquired in the negative ionization mode, and the total run time was 17 min (5–12.3 min in the 2nd segment and 11.6–22 min in the 3rd segment).

The selected reaction monitoring (SRM) transitions for each compound are also reported in Table 1. The first and more abundant was used for quantification (Q-SRM) and the second for confirmation (C-SRM). Besides retention time, the relative abundances of these selected SRM transitions were used to identify the metabolites in the samples and to discriminate against possible coelutions. Thus, for ion ratios (IRs = Q-SRM/C-SRM) of the standards between 1 and 2, DEAMPY, IMPY, TCPY, 3PBA and 4F3PBA, the IRs of the samples should not differ by >20%. For the metabolites with IRs between 2 and 5, MDA and CMHC, the ranges in the samples should not be lower than 25% and for those with IR between 5 and 10, PNP, lower than 30%. The resulting separation and selective MS traces for the organophosphate and pyrethroid pesticide metabolites are shown in Fig. 2.

Data adquisition, data handling and instrument control were performed with Masslynx software version 4.1 (Waters Inc., 2008). This software included the MRM tool that generates small dynamic periods or segments of acquisition around the expected retention time of the analyte of interest. The algorithm optimizes the dwell time based on the number of transitions that are co-eluting.

2.4. Quality assurance procedure

Synthetic urine (Surine, Preserve Free, Sigma-Aldrich) was used for blanks, quality control (QC) materials and standard preparation. Blanks were analysed for every set of 10 to 15 urine samples and were used for measuring of the existing contamination of the laboratory environment, including the material and solvents. Two synthetic urine samples fortified with the analytes were prepared at low (QCL, 1 μg/l) and high (QCH, 10 μg/l) concentrations. Calibration straight lines were prepared by adding 25 μl of standard solutions at concentrations ranging from 2.5 to 800 ppb into 1 ml synthetic urine sample, yielding final concentrations of 0.06, 0.12, 0.24, 0.48, 0.95, 1.9, 2.4, 4.8, 9.5, 13.3 and 19 ng/ml in urine. Quantification was performed by isotopically-labeled internal standards.

In addition, the methodology was externally checked out by participation in rounds of the German External Quality Assessment Scheme since 2016 (GEQUAS, 2016), which include the organophosphate metabolites PNP and TCPY and the pyrethroid metabolite 3-PBA.

Table 3
Results obtained in the analysis of proficiency testing materials from the G-Equas program. Reference values and tolerance ranges provided by G-Equas are also shown.

		PNP[a]	TCPY[a]	3-PBA[a]
RV-57	Result A	29	5.4	2.3
	Ref. value A	25.7 [19.1–32.3]	6.3 [4.8–7.9]	2.1 [1.7–2.5]
	Result B	160	10	6.9
	Ref. value B	151.4 [132.2–170.6]	12.2 [9.7–14.8]	6.1 [4.8–7.3]
RV-58	Result A	16.7	3.4	1.3
	Ref. value A	16.2 [12.6–19.8]	4.6 [3.4–5.8]	1.1 [0.89–1.3]
	Result B	52	11	3.7
	Ref. value B	51.7 [42.1–61.3]	15.2 [11.6–18.9]	3.5 [2.9–4.0]

[a] Acronyms in Table 1.

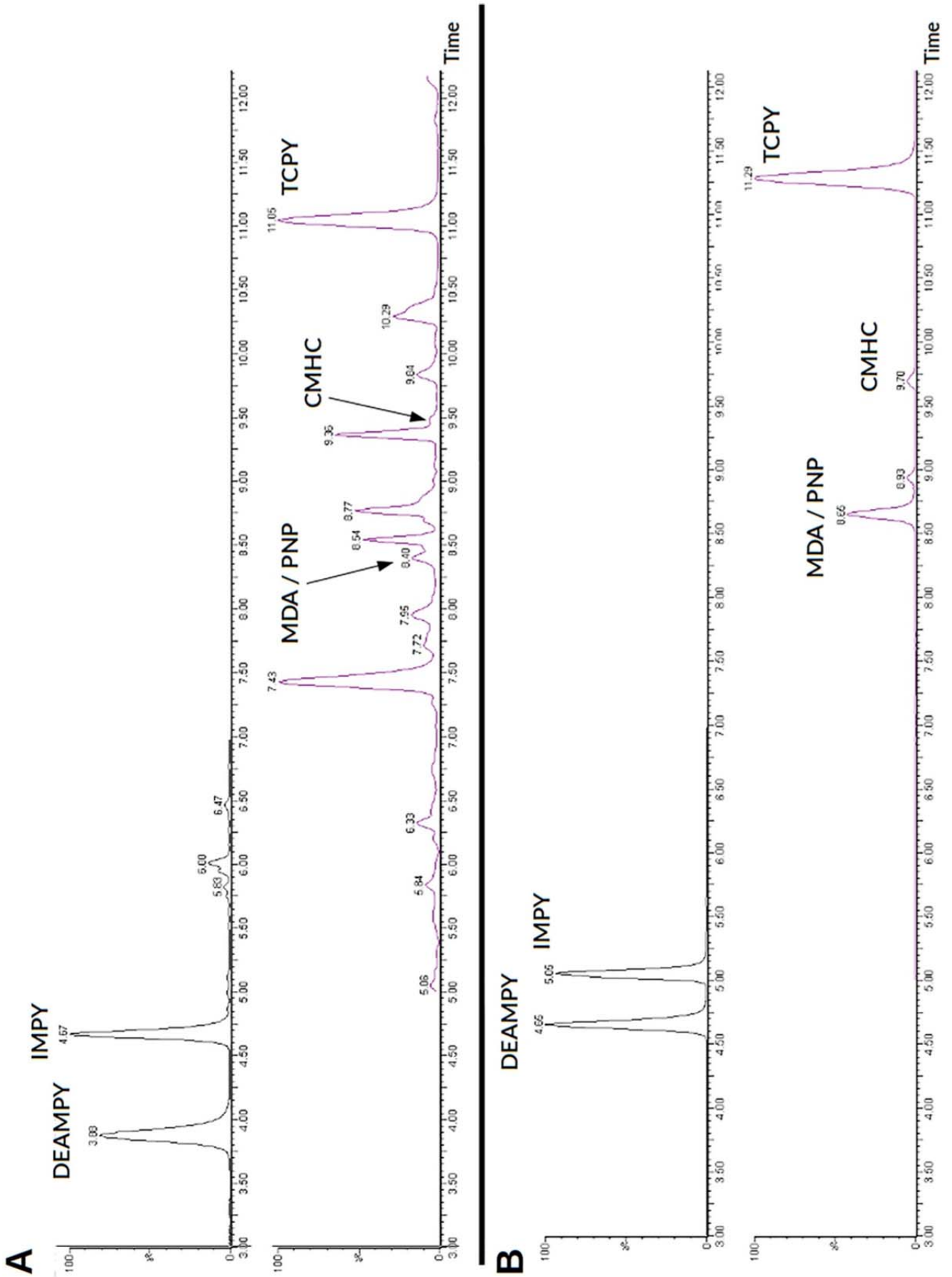

Fig. 3. TIC chromatograms of a real urine extract (A) and synthetic urine extract (B), for each of the three timed segments.

Limits of detection were calculated by the DIN 32645 methodology (equivalent to ISO 11843) using calibration straight lines. The calculation was carried out following previous descriptions (Massart et al., 1997) and implemented through chemCal package (Ranke, 2015) of the statistical software R (R Core Team, 2016).

2.5. Biological samples

Human urine samples ($n = 125$) from two adult Spanish populations in Catalonia and Galicia were analysed. One third of the cohort ($n = 48$) are farmworkers whose exposure levels to pesticides was presumably high, whereas the rest of the samples belonged to inhabitants from rural and urban areas located in Catalonia and Galicia. The samples were frozen within 4 h of collection and were stored at −20 °C until analysis.

3. Results and discussion

3.1. Method optimization

Precondition and elution of SPE cartridges with adequate solvents is a key step for the extraction procedure. The present optimized methodology used a mixture of methanol and acetone (25:75 v/v) for these steps (Fig. 1). Previously reported methodologies using SPE cartridges only used methanol (Olsson et al., 2004; Baker et al., 2004) or acetone (Davis et al., 2013), but not a mixture of both. In the present study, this mixture was found to be more effective for achieving better recoveries of certain metabolites, *e.g.* CMHC, 3-PBA and 4-F-3-PBA, without compromising the rest of the analytes. In addition, the same mixture was used for final vial reconstitution before injection (Davis et al., 2013).

Various mobile phases and other parameters (flow rate, solvation and column temperatures) were tested in order to achieve a good separation and peak shape for all target metabolites, posing special attention to MDA, which usually involves high difficulties. Previous studies have reported high limits of detection and low levels of relative recoveries for this compound (Davis et al., 2013; Olsson et al., 2004; Roca et al., 2014a, 2014b). Common solvents, including methanol and acetonitrile, and modifiers such as acetic acid (at different percentages ranging from 0% to 5%), ammonium acetate and ammonium formate (both at 20 mM) were tested, in combination with different gradient, flow rates (ranging from 0.2 to 0.5 ml/min), solvation temperatures (500 °C and 600 °C) and column temperatures (30 °C to 50 °C). Finally, a mobile phase containing HPLC H_2O with 5% methanol and 1% of acetic acid was the one which provided the best peak shape for MDA without compromising the analytical performance of the rest of the pesticide metabolites. Solvation temperature was set at 500 °C, the columnn temperature at 30 °C and a flow rate at 0.3 ml/min.

3.2. Method validation

Limits of detection were calculated from the calibration straight lines following the IUPAC recommendations (Massart et al., 1997; Currie, 1997). These calibration straight lines provided good linearity for all the compounds ($R^2 > 0.99$). Limits of detection below 0.069 μg per litre urine for OP metabolites and between 0.018 and 0.019 μg/l for PYR metabolites could be achieved (Table 2). These values were lower than those reported in previous similar methodologies (Roca et al., 2014b; Davis et al., 2013; Olsson et al., 2004). In addition, calculation of the LDs according to this method provides values that are close to the real limits when using synthetic urine (see next subsection below).

Accuracy and precision were assessed from two concentration levels, 1 ng/ml and 10 ng/ml, QCL and QCH, respectively, using synthetic urine. Recoveries ranged between 66% and 100% for CMHC and 4F3PBA, respectively (Table 2). The repeatability and reproducibility coefficients of variance (CV) were lower than 20% in all cases and lower than 10% in the repeatability runs (Table 2).

Analysis of proficiency testing materials obtained from the G-Equas programme (GEQUAS, 2016) provided results within the range of 20% of the consensus values (Table 3).

3.3. Matrix effects

Matrix effects are common in urine analyses which are already observed as interferences in the calibration straight lines (Tudela et al., 2012; Deventer et al., 2014). Human urine samples diluted in water are generally used for the preparation of these calibration straight lines (Olsson et al., 2004; Davis et al., 2013; Roca et al., 2014a, 2014b). However, human urine composition varies greatly due to many different factors. In the present study, the use of synthetic urine has been observed to be the best method to solve these interfering matrix effects. This approach is also useful to account for possible contaminations when used for blanks. A total of 10 procedural blanks of synthetic urine were analysed. Overall, the concentrations of the analysed metabolites, when found in blank urines, corresponded to small contamination of the analytical process and did not biased the final results. The total ion chromatograms (TIC) of real and synthetic urine extracts previously fortified with the analytes of interest are compared in Fig. 3. As shown in this figure, real urine extracts present many peaks, specially in the second segment, which may interfere in the real signals of calibration straight lines for specific compounds, *e.g.* MDA, PNP, CMHC and TCPY, whereas fortified sythetic urine samples only show the peaks of the selected analytes.

3.4. Analysis of real samples

The application of this procedure to human urine samples from rural farmers and general population living in rural and urban areas shows that PNP (found in all samples analysed), TCPY (found in 95% of the samples) and DEAMPY (77%) were the most abundant OP metabolites, with median concentrations of 1.8 ng/ml, 1.1 ng/ml and 3.2 ng/ml, respectively (Table 4). None of the samples showed MDA, and a few of them (<5%) had detectable concentrations of IMPY and CMHC (Table 4). Concerning the PYR metabolites, 3-PBA was found in 81% of the samples (median 1.5 ng/ml) and 4-F-3-PBA

Table 4
Detection frequencies (DF, %) and median, mean and concentration ranges (ng/ml) of organophosphate and pyrethroid pesticide metabolites in urine of adults from Catalonia and Galicia.

	Total ($n = 125$)				Farmworkers ($n = 45$)				Rural & urban ($n = 80$)			
	DF (%)	Median	Mean	Range	DF (%)	Median	Mean	Range	DF (%)	Median	Mean	Range
DEAMPY[a]	77	1.1	2.2	nd – 18.8	82	1.7	2.9	nd – 15.6	74	0.81	1.9	nd – 18.8
IMPY	2	nd	0.24	nd – 14.2	2	nd	0.32	nd – 14.2	3	nd	0.20	nd – 13.7
MDA	0	–	–	–	0	–	–	–	0	–	–	–
PNP	100	1.8	2.9	0.059–16.0	100	2.3	3.9	0.25–16.0	100	1.3	2.3	0.059–14.8
CMHC	1	0.013	0.014	nd – 0.10	0	–	–	–	1	nd	0.014	nd – 0.10
TCPY	95	3.2	3.7	nd – 20.0	100	4.2	5.4	1.2–20.0	93	2.2	2.7	nd – 8.8
3-PBA	82	1.5	2.5	nd – 20.5	91	2.4	3.7	nd – 20.5	76	1.1	1.8	nd – 15.0
4-F-3-PBA	54	0.076	0.088	nd – 0.34	47	nd	0.084	nd – 0.26	58	0.079	0.091	nd – 0.34

[a] Acronyms in Table 1.

was found in half of the cohort (54%) with median concentrations of 0.076 ng/ml (Table 4).

Comparison of the population of farmworkers with the population of non-farmworkers living in rural and urban areas shows higher concentrations of DEAMPY (medians 1.7 *vs.* 0.81 ng/ml, respectively), PNP (2.3 *vs.* 1.3 ng/ml), TCPY (4.2 *vs.* 2.2 ng/ml) and 3-PBA (2.4 *vs.* 1.1 ng/ml) in the former (Table 4). These differences are consistent with occupational activity (Ye et al., 2013; Wang et al., 2016; EFSA, 2014). Farmworkers are directly exposed to these pesticides through inhalation, dermal contact and indirect ingestion (*e.g.* skin, eyes), through the manipulation of these substances when either mixing, loading and handling treated crops, or spraying and applying them into the fields (Egeghy et al., 2011; EFSA, 2014). A continuous exposure can occur if workers and operators do not undertake additional measures during and after work, including use of personal protective equipment (*e.g.* gloves, mask, glasses) or washing regularly contaminated clothing (Arcury et al., 2009; Farahat et al., 2011). However, the aforementioned median values show differences of two times, indicating that people not occupationally exposed to the use of these pesticides is also incorporating these compounds, probably as consequence of food consumption. This is consistent with previous studies in which OP and PYR metabolites were positively associated with higher intakes of fruits and vegetables (Llop et al., 2017). The studied pesticides are commonly used in agriculture, and some of them have been encountered in food products from European countries (EFSA, 2017). For instance, the OP pesticide chlorpyriphos (metabolized into TCPY in humans) is one of the most frequently found pesticides in plant products, and the one with higher number of quantifications exceeding the maximum residue levels (MRL) allowed by the EU legislation (EFSA, 2017).

OP and PYR pesticides are also employed as biocidals for domestic purposes, for household pets and gardening, among other uses (*e.g.* ornamental plants), and exposure of general populations through other non-food sources in rural and urban areas should not be underestimated. In addition, residents living in areas close to the application of pesticides may be at increased risk of exposure, in a similar way than occupationally-exposed individuals (EFSA, 2014).

The present study is the first comparing the metabolite OP and PYR concentrations among general and occupationally exposed populations in countries with strong agricultural activities such as those in Spain. The results show that both populations are generally exposed to pyrethroids and OP pesticides, including chlorpyriphos, pirimiphos and parathion.

4. Conclusions

The isotope dilution solid phase extraction UPLC-MS/MS method developed in the present study is adequate for the analysis of organophosphate and pyrethroid pesticide metabolites in urine samples from general and potentially highly exposed human populations. It allows the determination of eight target pesticide metabolites in urine with satisfactory sensitivity, accuracy and precision. The use of methanol:acetone (25:75 v/v) for extraction is more effective and provides better recoveries than these individual solvents. In addition, the use of synthetic urine improves significantly the method selectivity and limits of detection in the construction of the calibration straight lines. Detection limits in the range of 14–69 pg/ml were obtained. Metabolites of both pesticide groups were observed in both occupationally and non-occupationally exposed populations, the former showing twofold average concentrations of organophosphate metabolites than the second.

Acknowledgements

Financial support was has been received from the EU projects HEALS (FP7-ENV-2013-603946) and NEUROSOME (H2020-MSCA-ITN-2017 SEP-210411486), and the Spanish GRACCIE-Redes project (CTM2014-59111-REDC).

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ARTICLE 5

Urinary metabolites of organophosphate and pyrethroid pesticides in children form an Italian cohort (PHIME, Trieste).

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Environmental Research 176, 108508 (2019)

DOI 10.1016/j.envres.2019.05.039

Environmental Research 176 (2019) 108508

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Urinary metabolites of organophosphate and pyrethroid pesticides in children from an Italian cohort (PHIME, Trieste)

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ARTICLE INFO

Keywords:
Organophosphate pesticides
Pyrethroids
Human biomonitoring
Children
Estimated daily intakes

ABSTRACT

Urinary metabolites of organophosphate (OP) and pyrethroid (PYR) pesticides from seven years old children of a birth cohort study (n = 199; PHIME cohort of Trieste, Italy) have been measured. Six OP and two PYR metabolites have been investigated, 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY, pirimiphos metabolite) was the one found at higher concentrations, median 3.4 ng/mL specific gravity adjusted (SG adjusted), followed by 4-nitrophenol (PNP, median 1.4 ng/mL SG adjusted) and 3,5,6-trichloro-2-pyridinol (TCPY, median 0.36 ng/mL SG adjusted), parathion and chlorpyriphos metabolites, respectively. TCPY concentrations were low in comparison to other distributions of OP metabolites in children from other studies. Accordingly, the PHIME cohort showed a distinct OP metabolite distribution with high concentrations of pirimiphos and parathion. Another specific characteristic of this cohort was the high concentration of 3-phenoxybenzoic acid (3-BPA, median 0.36 ng/mL SG adjusted), a general metabolite of PYR pesticides.

Evaluation of anthropometric and socio-demographic characteristics of children and families only showed a positive association between family educational level and urinary concentrations of DEAMPY metabolite ($p < 0.05$), which could reflect distinct dietary habits depending on the educational level. Estimated daily intakes were evaluated, all studied metabolites were found within safe levels.

1. Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture and for domestic and gardening use. They have been designed to eliminate insects but they can also affect non-targeted species, including humans, causing them adverse health effects (Barr, 2008). The PYR insecticides were developed as a synthetic version of the naturally occurring pesticide pyrethrin. They are currently replacing OP and carbamate insecticides in domestic and agricultural use as they are safer for the mammalian species (Narahashi et al., 2007).

Nevertheless, public concern on pesticide use is increasing, not only for the negative impacts on wildlife and environment but also for the potential health effects on humans. OP and PYR pesticide exposure has been related to respiratory, digestive, reproductive and neurological problems, among others (Ye et al., 2017; Arcury et al., 2016; Llop et al., 2017).

Lately, these chemicals have been found in different matrices, including dietary products, water, outdoor and indoor air and house dust (Banerjee et al., 2012; Sousa et al., 2018; Coscollà et al., 2017; Gibbs et al., 2017; Mercier et al., 2011; Tang et al., 2018). In addition, recent studies have found them even in freshwater and edible fish (Arisekar et al., 2019; Barbieri et al., 2019; Pico et al., 2019), being in some cases related with pollution accidents, like the death fish episode by pyrethroid exposure in Northern Italy (Bille et al., 2017).

Exposure to OPs and PYR pesticides is thought to occur via

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https://doi.org/10.1016/j.envres.2019.05.039
Received 13 October 2018; Received in revised form 13 May 2019; Accepted 23 May 2019
Available online 29 May 2019
0013-9351/

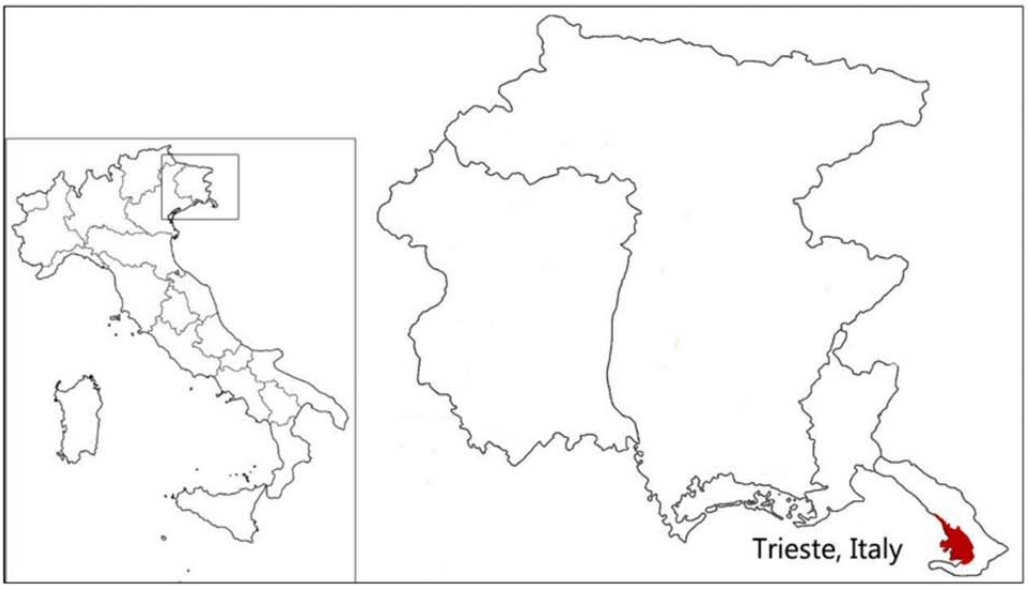

Fig. 1. Sampling location. Trieste, Italy.

ingestion, direct skin contact in domestic uses and inhalation by proximity to spraying areas. Diet has been identified as the primary source of exposure in the general population (Becker et al., 2006; McKone et al., 2007). Several studies have recently investigated the influence of various foods and food groups (Fortes et al., 2013; Lewis et al., 2015; Jardim et al., 2018).

When OPs and PYR enter human body, they are metabolized and then excreted in the urine, either in free form or bound to glucoronic acid or sulfates (Barr, 2008). A two steps metabolic pathway transforms OPs into dialkyl phosphate (DAP) and hydroxylated organic moieties that are specific of each pesticide (Chambers and Russel, 1995). The metabolic reactions of pyrethroids proceed in two steps, involving the generation of an acid and an alcohol moiety per compound which are excreted (Mikata et al., 2011).

Children are especially susceptible to environmental toxicants such as OP and PYR pesticides due to their developmental immaturity. Their organs are more vulnerable (particularly the brain and nervous system), they have lower capacity to absorb and eliminate chemicals and they are exposed at higher levels, increasing their risk, compared with adults (National Research Council, 1993; Landrigan et al., 2004, 2019; Katsikantami et al., 2019). The discovery of OPs and PYR pesticides in amniotic fluid and meconium (Berton et al., 2014; Bradman et al., 2003) indicated that foetuses are already exposed to these chemicals. After birth, babies and toddlers can be also exposed via breastfeeding, repeated hand-to-mouth ingestion, diet and ambient or airborne exposure (Zartarian et al., 2000; Weldon et al., 2011; Yusa et al., 2015). Early life exposure may be particularly detrimental given the rapid and formative brain development occurring during these periods (Eskenazi et al., 2014). In addition, children younger than seven years of age do not have adult levels of enzymes to detoxify OP pesticides (Huen et al., 2009).

There is an increasing evidence of the relationship between OP pesticide and PYR exposure and health effects. Adult exposure has been associated with various adverse health outcomes, including cancer (Engel et al., 2017), impacts on the reproductive and endocrine systems (Kamijima et al., 2004; Ram, 2017; Lerro et al., 2018) or diabetes (Starling et al., 2014; Park, et al. 2019). Prenatal and postnatal exposure has also been linked with children health effects. The former has been associated with neurodevelopmental problems (Gonzalez-Alzaga et al., 2014; 2015), low birth weight, increased child blood pressure (Harari et al., 2010), shorter time of gestation (Eskenazi et al., 2004), respiratory outcomes (Reardon et al., 2009, Raanan et al., 2016), obesity and diabetes (Bost-Legrand et al., 2016; Slotkin, 2011). The latter was found to affect negatively neurodevelopmental outcomes, such as working memory, attention or motor speed (Ruckard et al., 2004; Rohlman et al., 2005, Oulhote and Bouchard, 2013; Cartier et al., 2016). It is worth noting that adverse OP effects, which include cognitive impairment and attention deficit, occur at levels well below those causing inhibition of blood cell AcHE. These levels are still considered as toxicological end point references in risk assessment to establish adverse daily intakes (ADIs) in humans (for an updated review see Hertz-Picciotto et al., 2018).

These results evidence the need for a more detailed assessment of the effects to these compounds during all human age periods but particularly in the early life stages. This information may also be useful for designing adequate remediation actions towards the minimization of the exposure to these pesticides, particularly in the first years of age.

Unfortunately, the information available on the occurrence of these pesticides in children is scarce. The present study is aimed to contribute to fill this gap by analysis of urine from 7 years old children in Trieste (Italy). Pesticides are one of the most frequently detected classes of pollutants in Mediterranean countries such as Italy (Meffe and de Bustamante, 2014) due to their widespread use, particularly in areas of extensive agriculture. According to the Statistical Office of the European Union, Italy is one of the countries with the highest use of pesticides in Europe (Eurostat, 2014).

2. Methods

2.1. Population and study design

The present work is focused on 7 year-old children, who belong to the Italian prospective mother-child cohort, Northern Adriatic Cohort II (NAC II), of the "Public health impact of long-term, low-level mixed element exposure in susceptible population strata" (PHIME) project.

The aim of PHIME was to assess the association between mercury exposure from food consumption during pregnancy and development of the children nervous system at 18 months. A detailed description of the study protocol has been published previously (Valent et al., 2013). In brief, 900 pregnant women permanent residents in the study area for at least 2 years, having 18 years of age or more, and not having been absent from the study area for more than 6 weeks during pregnancy

were recruited between 2007 and 2009 at the Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, Italy (Fig. 1).

The follow-up of the Italian children born within the NAC II cohort of PHIME proceeded until children's 7 years, with the purpose of assessing the effect of low level mercury exposure through foods and in particular through fish consumption on the developing nervous system at this age. Participant subjects were the 767 children born within the NAC II cohort of PHIME between April 2007 and April 2009 and reaching the 7 years old between 2014 and 2016. Children whose parents gave consent to be involved in the NAC II cohort of PHIME and in its continuation were included in the study. The 7 years old follow-up took place at the Institute for Maternal and Child Health IRCCS Burlo Garofolo in Trieste, Italy.

The present work involved a subset of 199 children followed up between August 2014 and December 2015, within the "Crome Life-Cross Mediterranean Environment and Health Network" project.

The research protocol was approved by the ethics Committee of the University of Udine and of the Institute for Maternal and Child Health IRCCS Burlo Garofolo in Trieste (Italy).

2.2. Sample collection and questionnaires

Children urine at 7 years were collected in Kartell™ bottles by mothers at home and then brought in to research personnel. Successively, urine samples were transferred, by research personnel, into Falcon polypropylene tubes and stored in freezer (initially at −20 °C and then at −80 °C).

Socio-demographic information was obtained through questionnaires administered to parents during the pregnancy period (Valent et al., 2013) and when children were 7 years old. The variables used in this study were: children's gender and BMI and parents' educational level and if they smoke or not at home, at child age of 7 years.

Family dietary habits were collected after delivery by administration of a questionnaire to the mothers which concerned consumption of 138 food items and was adapted from a previously validated food frequency questionnaire (Franceschi et al., 1993, 1995; Decarli et al., 1996). Supplementary questionnaires were administered when children were eighteen months and seven years old, respectively. These questionnaires assessed changes in anthropometric measures and developmental milestones of the child, breastfeeding history, child fish intake, diseases and daycare attendance. Regarding fish intake, servings per week were transformed into amounts considering one serving as 150 g and half serving as 80 g. Four different kinds of fish were taken into account: fresh fish, shellfish, clams and canned fish.

2.3. Sample preparation and instrumental analysis

Sample preparation and analysis follows the method described in Garí et al. (2018). Briefly, centrifuged and filtered urine samples (1 mL) were introduced into 10 mL centrifuge tubes for hydrolysation with β-glucoronidase. A mixture of the available isotopically labelled internal standards (25 μL) was also added. The hydrolysed samples were cleaned up by solid-phase extraction (SPE). The SPE cartridges (Oasis HLB 3 cm^3, 60 mg) were preconditioned with a mixture of MeOH:Acetone (25:75 *v*/v) followed by H_2O containing 1% acetic acid. The OP and PYR metabolites were eluted with 1.5 mL of MeOH:Acetone (25:75 *v*/v). The collected extracts were reduced under N_2 to near dryness and transferred to chromatographic vials with 120 μl of MeOH:H_2O (25:75 *v*/v).

Identification and quantification of six specific OP metabolites and the 2 PYR biomarkers showed in Table 1, was carried out by isotope dilution solid phase extraction UPLC-MS/MS using an Ultra-Performance Liquid Chromatography (UPLC Acquity H-Class, Waters, Milford, MA, USA) coupled to a Triple Quadrupole Mass Spectrometer (XEVO-TQ-S, Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) interface. The chromatographic separation was performed on a Betasil C18 column (100 mm x 2.1 mm, 3 μm particle size). The injection volume was 10 μl, at a flow rate of 0.3 mL/min. The column temperature was kept at 30 °C during the analysis. A gradient elution with a mobile phase of acetonitrile and a mixture of H_2O with 1% acetic acid and 5% methanol was used. Target compounds were positively identified by their retention times and the ratio of the two MRM transitions, which had to fall within ± 20% of the average ratio obtained from standard solutions.

Synthetic urine (Surine, Preserve Free, Sigma-Aldrich) was used for blanks, quality control (QC) materials and calibration curves. Accuracies were assessed at two levels (QCLow, 1 μl/L and QCHigh 10 μl/L). Calibration curves were prepared by adding 25 μl of standard solutions at concentrations between 2.5 and 800 ppb into 1 mL of synthetic urine. The quantification was performed using the isotopically-labelled internal standards (Garí et al., 2018). This methodology was additionally checked out by participation in rounds of the German External Quality Assessment Scheme since 2016 (G-EQUAS), which includes the OP metabolites PNP and TCPY and the 2 PYR pesticide metabolites 3-PBA and 4-F-3-PBA, providing results within the range of 20% of the consensus values. To our knowledge there is no proficiency testing program for the other analytes or a program including all the analytes from the present study.

Specific gravity (SG) was measured in all urine samples. This parameter ranged between 1.001 and 1.020 g/mL (mean = 1.0077 g/mL) and was used to normalize the measured individual metabolite concentrations to the general dilution pattern of the whole cohort. The SG corrected concentrations were obtained by applying the formula: $[OPs/PYR]^i_{SG} = [OPs/PYR]^i * (\bar{x}_{SG}-1)/(SG^i-1)$ where $\bar{x}_{SG}$ is the average specific gravity of the cohort, $[OPs/PYR]^i$ is the measured concentration of the i individual, and SG^i is the specific gravity of the urine of i (Boeniger et al., 1993).

2.4. Calculation of OP and PYR daily intakes

Biomonitoring studies present concentrations of biomarkers in biological matrices; however, pesticide regulation committees (European Comission) indicate the maximum acceptable doses as acceptable daily intakes (ADI) and reference dose (RfD) expressed as μg/kgbw/day. Thus, OPs and PYR daily intakes were estimated for the analysed pesticides based on the molar levels of the urinary metabolites, using the following toxicokinetic model by Katsikantami et al., 2019:

$$EDI\left(\frac{\frac{\mu g}{kg\ bw}}{d}\right) = \frac{C_U\left(\frac{\mu mol}{L}\right) * V_U(L) * MW_P\left(\frac{g}{mol}\right)}{F_{UE} * BW\ (kg)}$$

where EDI is Estimated Daily Intake, C_U the molar concentration of metabolite, V_U the total urinary volume excreted within 24 h, MW_P the molecular weight of the parent compound, F_{UE} the urinary excretion factor of the parent compound and BW the body weight.

Data used for the calculation are shown in Table 2, 24-h urine volumes were estimated as 0.82 L (Cequier et al., 2017). Molecular weight for pirethroids with 3-PBA as main metabolite was estimated as the mean of the most common pirethroids (permetrin, deltametrin, fenvalerate and cypermetrin). Excretion factor data were available for all metabolites except DEAMPY and only applicable to adults, hence two approximations were done, one using the found F_{UE} and the other assuming a minimum of 5 and a maximum of 100% of excretion.

2.5. Data analysis

Data analysis and graphics were performed using the statistical software R (R Development R Core Team, 2018). The plyr and ggplot R packages were used. Statistics were focused on the metabolites found above the limit of detection (LOD) in more than 20% of the samples: DEAMPY, PNP, TCPY, 3-PBA and 4-F-3-PBA. One-half of the LOD was assigned to non-detected values.

Geometric mean (GM) and its 95% confidence interval (CI), median

Table 1
Metabolite's acronyms and principal uses of the pesticides analysed.

Acronym	Analyte	Pesticide	Principal uses	Status[a] (legislation)
DEAMPY	2-diethylamino-6-methylpyrimidin-4-ol	Pirimiphos	All crops, especially fruits and citrus plantations and agricultural facilities	Approved[b]
IMPY	2-isopropyl-6-methyl-4-pyrimidol	Diazinon		Not approved (2007/393)
MDA	Malathion dicarboxylic acid	Malathion		Approved
PNP	4-nitrophenol	Parathion		Not approved (01/520/EC)
TCPY	3,5,6-trichloro-2-pyridinol	Chlorpyriphos		Approved
CMHC	3-chloro-4-methyl-7-hydroxycoumarin	Coumaphos	Farm and domestic animals to control mite	Not approved[c]
3-PBA	3-phenoxybenzoic acid	Common pyrethroids	Parks and gardens, forestry plantations, agricultural crops, pets and lice	Approved[d]
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid	Cyfluthrin		Not approved (460/2014)

[a] European Commission.
[b] Pirimiphos-ethyl is not approved (2002/2076).
[c] Never notified and authorised in EU.
[d] Approved pyrethroids: Cypermethrin, deltamethrin, esfenvalerate or etofenprox.

Table 2
Data used for the calculation of children's EDIs.

	MW_M (g/mol)	MW_P (g/mol)	F_{UE}	Reference
DEAMPY	181.23	305.33	–	
IMPY	152.19	304.35	0.6	Payne-Sturges, 2009
PNP	139.11	291.26	0.36	Morgan et al., 1977
TCPY	198.43	350.57	0.7	Payne-Sturges, 2009
3-PBA	214.22	462.92	0.24	*Various authors*[a]
4F-3-PBA	232.21	434.30	0.4	Leng et al., 1997

MW_m, Metabolite molecular weight; MW_P, Parent compound molecular weight; F_{UE}, Urinary excretion factor; [a]Woollen et al., 1992, Sams and Jones, 2012, Ratelle et al., 2015

and interquantile ranges were used for descriptive analysis. Mann Whitney U test was used to evaluate concentration differences between the socio-demographic groups.

Multivariate models were performed to evaluate the relations of sociodemographic factors and child's fish consumption with pesticide concentration, compound concentrations were transformed into the natural logarithms because of its skewed distribution.

3. Results

3.1. Socio-demographic characteristics

Table 3 summarizes the descriptive characteristics of the 199 families included in this study. Thirty-eight percent of children were boys and 62% girls, their average weight and height were 25.7 kg and 123.9 cm, respectively. Concerning BMI, most of the children had normal weight (69%), while 19 and 12% were overweight or obese, none of the children were classified as underweight (WHO, 2007).

The highest educational level of one of the parents was chosen for family classification. Due to the uneven distribution of the five categories (Table 3), the participant families were grouped into two categories, with and without university studies (n = 92, 46% and n = 106, 54%, respectively). Concerning home smoking, the families were included in the "smoking group" if one of the parents was smoker (63%). Non-smoking families were 37%.

3.2. Urinary concentrations of OP and PYR metabolites

Descriptive statistics for the measured urinary concentrations of the analysed metabolites are summarized in Table 4. Detection frequencies (DFs; > LOD) of each compound ranged from below LOD to 97% of detection, being PNP (DF = 97%) and DEAMPY (DF = 96%) the compounds found most frequently above the LOD, followed by TCPY (detected in 80%), and the PYR pesticide metabolites, 3-PBA and 4F-3-PBA, with DF of 81% and 24%, respectively.

The sum of the three most abundant OP metabolites, DEAMPY, PNP, TCPY, ranged between 0.018 and 73 ng/mL SG adjusted with a median of 8.0 ng/mL SG adjusted. The median of the sum of PYR metabolites, 3-PBA and 4-F-3-PBA, was more than ten times lower compared with the sum of OPs, 0.71 ng/mL SG adjusted, ranging between below the limit of detection and 69 ng/mL SG adjusted.

The most abundant OP pesticide was DEAMPY, the metabolite of pirimiphos (median 4.5 ng/mL SG adjusted), followed by PNP and TCPY, the metabolites of parathion and chlorpyriphos, respectively, with medians of 1.5 and 0.41 ng/mL SG adjusted. Regarding pyrethroids, 3-PBA metabolite was the one found at highest concentration with a SG adjusted median of 0.57 ng/mL against 0.015 ng/mL of 4-F-3-PBA. The higher concentration of 3-PBA is expected since it reflects contributions from several pyrethroids whereas 4-F-3-PBA is specific of cyfluthrin.

Median values, interquartile range and results obtained after using the Mann-Whitney U-test for two independent samples are shown in Table 5. There are no significant differences with respect to children gender and BMI or if parents smoke at home or not (Fig. S1. Supplemental material). However, children with at least one parent with higher educational level (university degree) had higher concentrations of DEAMPY metabolite (Table 5).

Multivariate linear regression analysis adjusted by children's BMI, gender and family education was performed to explore the influence of fish consumption on the OP and PYR concentrations (Table S1. Supplemental material). Fish intake was categorized between children who had 1 or more servings per week and those having less than 1. Fish

Table 3
Socio-demography of the study participants.

	Children
All participants	199 (100)
Gender (n = 198)	
Male	76 (38)
Female	122 (62)
Weight (n = 178)	25.7 ± 4.7
Boys (n = 66)	25.6 ± 3.7
Girls (n = 112)	25.8 ± 5.2
Height (n = 178)	123.9 ± 5.1
Boys (n = 66)	124.0 ± 4.2
Girls (n = 112)	124.0 ± 5.6
BMI (n = 178)	
Normal	122 (69)
Overweight	34 (19)
Obese	22 (12)
Family education (n = 198)	
Elementary	1 (1)
Middle	16 (8)
High school	89 (45)
University	92 (46)
Smoking at home (n = 194)	
Yes	71 (37)
No	123 (63)

Table 4
Specific gravity (SG) adjusted and non-adjusted urinary levels (ng/mL) of OP and PYR metabolites. Full names of the metabolites in Table 1.

Metabolite	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	DF[c] (%)	SG adjusted results (n = 198)				Non-adjusted results (n = 199)			
				GM	(CI)[c]	Median	Range	GM	(CI)[c]	Median	Range
DEAMPY	0.017	0.025	96	3.4	(2.6-4.5)	4.5	< LOD -71	2.7	(2.1-3.5)	3.0	< LOD -110
IMPY	0.014	0.021	6	< LOD		< LOD	< LOD -0.50	< LOD		< LOD	< LOD -0.98
MDA	0.069	0.10	0	< LOD		< LOD	–	< LOD		< LOD	–
PNP	0.017	0.025	97	1.4	(1.1-1.7)	1.5	< LOD -37	1.1	(0.92-1.3)	1.2	< LOD -14
TCPY	0.020	0.039	80	0.29	(0.22-0.38)	0.41	< LOD -30	0.23	(0.18-0.30)	0.36	< LOD -6.0
CMHC	0.026	0.030	1	< LOD		< LOD	< LOD -0.96	< LOD		< LOD	< LOD -0.25
3-PBA	0.018	0.027	81	0.36	(0.28-0.48)	0.57	< LOD -69	0.29	(0.22-0.38)	0.56	< LOD -36
4F-3-PBA	0.019	0.028	24	0.027	(0.022-0.034)	0.015	< LOD -3.0	0.022	(0.018-0.027)	< LOD	< LOD -1.3
ΣOP[d]	–	–	91	7.4	(6.1-9.1)	8.0	0.018-73	5.9	(5.0-7.0)	6.6	0.026-120
ΣPYR[e]	–	–	53	0.55	(0.44-0.69)	0.71	< LOD -69	0.43	(0.35-0.54)	0.59	0.019-36

[a] LOD: Limit of detection.
[b] LOQ: Limit of quantification.
[c] DF: Detection frequencies (> LD); GM (CI): Geometric mean and 95% confidential interval.
[d] ΣOP = [DEAMPY] + [PNP] + [TCPY].
[e] ΣPYR = [3-PBA] + [4-F-3-PBA]

consumption was not significant for the urinary OP and PYR concentrations.

3.3. Estimated daily intakes (EDIs)

Median EDIs with interquartile range (Q_1-Q_3) for children are presented in Fig. 2 in three different cases of urinary excretion factor (F_{UE}), ADIs are marked in red for each compound. The hypothetical worse-case for F_{UE} has been placed at 5% of excretion, the only metabolite crossing this threshold is PNP, parathion metabolite. Using the values for F_{UE} found in previous studies (Morgan et al., 1977; Wollen et al., 1992; Leng et al., 1997; Bouchard et al., 2003; Payne-Sturges et al., 2009; Sams and Jones, 2012; Ratelle et al., 2015), as mentioned before they are usually done with a very small population and just for adults, all the EDIs are below the ADIs, within safe levels, for all the studied pesticides.

4. Discussion

The present study combines the measurement of OPs (six metabolites) and PYR (two metabolites) to obtain a more comprehensive description of children exposure to pesticides.

4.1. Organophosphate pesticides

DEAMPY (metabolite of pirimiphos) is the most abundant OP in the present cohort, 3.0 ng/mL (Table 4), and a distinct feature when compared with the OP concentrations found in children of other studies (Table 6). The median of this metabolite was below LOD in Seattle and in Valencia (Lu et al., 2008; Roca et al., 2014) and below 1 ng/mL in North Carolina and Thailand (Arcury et al., 2007; Panuwet et al., 2009). Northern Italy has high levels of cereals production (Eurostat, 2016) and pirimiphos is an insecticide and acaricide used for the protection of stored grain but in our study it is not possible to define the source of exposure. However, a previous study analysing the occurrence and distribution of pesticides in the province Bologna (Northern Italy) found pirimiphos between the most detected compounds (Ghini et al., 2004).

TCPY (metabolite of chlorpyriphos), the dominant OP in the other cohorts, was also present in Trieste but at a median concentration, 0.36 ng/mL, much lower than the other studies, 2.5-3.7 ng/mL (Table 6). The Ministry of Health Report on Plant Protection Products in Food (Ministerio della Salute, 2018), based on data collected in Italy between 2015 and 2016, indicates among the pesticide residuals more frequently found in the Fruili Venezio-Giuglia Region, a significant presence of pirimiphos in cereals, as well as of chlorpyriphos and several pyrethroids in fruits.

Concerning other OP metabolites, PNP in the Trieste cohort, 1.2 ng/mL, was in the range of the concentrations observed in other studies, 0.93-2.9 ng/mL (Roca et al., 2014; Panuwet et al., 2009; Arcury et al., 2007). Despite the European banning of parathion since 2008, this is one of the OP found in higher concentrations in children from the compared cohorts. Given the reactivity of this compound and the low persistence in environmental samples, its rather high concentration

Table 5
Median urinary concentration differences (ng/mL SG adjusted) of principal metabolites detected by characteristics of study population.

Characteristics	n (%)	DEAMPY		IMPY		PNP		TCPY		3-PBA		4F-3-PBA	
		P50	IQR[a]	P50	IQR	P50	IQR	P50	IQR	P50	IQR	P50	IQR
Gender													
Boys	76 (38)	4.7	0.92-20	0.0089	0.0045-0.012	1.5	0.85-3.7	0.30	0.069-0.87	0.62	0.20-1.4	0.015	0.0073-0.077
Girls	122 (62)	4.5	1.3-10	0.011	0.0054-0.013	1.4	0.57-3.4	0.47	0.075-1.6	0.47	0.070-1.4	0.015	0.0085-0.077
BMI													
Normal	122 (69)	4.6	1.3-13	0.011	0.0054-0.013	1.4	0.56-3.8	0.4	0.044-1.2	0.65	0.069-1.6	0.015	0.0077-0.077
Overweight/Obese	54 (31)	3.2	0.82-11	0.0077	0.0041-0.011	1.7	0.75-3.2	0.47	0.16-1.1	0.48	0.16-0.95	0.015	0.0059-0.077
Family education													
Below university	106 (54)	**2.8***	**0.96-8.5**	0.0089	0.0045-0.013	1.3	0.52-3.2	0.43	0.059-1.4	0.49	0.069-1.0	0.015	0.0077-0.13
University degree	91 (46)	**6.1***	**1.5-16**	0.0089	0.0054-0.013	1.8	0.75-3.7	0.39	0.083-1.3	0.7	0.16-1.8	0.015	0.0077-0.038
Smoking at home													
Yes	71 (37)	3.4	0.82-11	0.0089	0.0045-0.013	1.6	0.59-4.2	0.37	0.054-1.1	0.41	0.17-1.2	0.015	0.0064-0.038
No	123 (63)	5.7	1.2-13	0.0089	0.0054-0.013	1.4	0.59-3.4	0.45	0.082-1.4	0.65	0.077-1.6	0.015	0.0096-0.12

[a] IQR: Interquartile range (P25-P75); * *p-value* for Mann-Whitney U test < 0.05

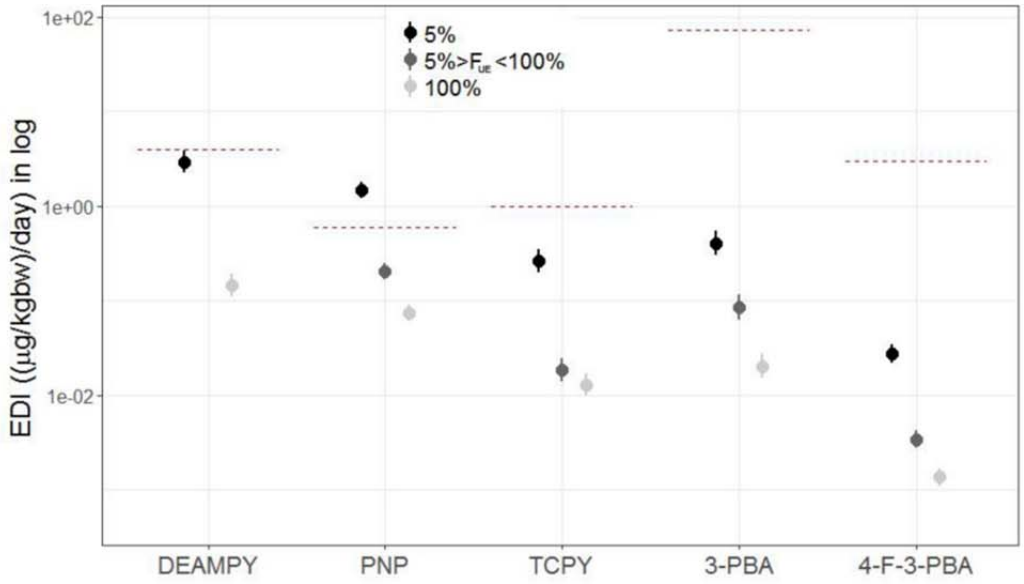

Fig. 2. Median estimated daily intakes with interquartile range (Q_3-Q_1) for children. In red, acceptable daily intakes (ADI). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 6
Comparison of the median urine concentrations in children from Trieste with those in children from other cohort studies (ng/mL). Full names of the metabolites in Table 1.

	N	Year	DEAMPY	IMPY	MDA	PNP	TCPY	CMHC	3-PBA	4-F-3-PBA	Reference
Italy (Trieste)	199	2014-2016	3.0 [2.7][b]	< LOD[a] < LOD	< LOD < LOD	1.2 [1.1]	0.36 [0.23]	< LOD < LOD	0.56 [0.29]	< LOD [0.022]	*Present study*
US (NHANES)	481	1999-2000			0.49		2.7 [2.88]				*Barr, D. B.* et al., *2005*
US (North Carolina)	60	2004	0.14	0.49 [0.56]	0.21	1.6 [1.0]	2.5 [1.9]	0.14	0.07		*Arcury, T. A.* et al., *2007*
US (Seattle)	23	2003-2004	< LOD	< LOD	1.6		3.7	< LOD			*Lu, C.* et al., *2008*
Thailand	207	–	0.14 [0.16]	< LOD	0.21 [0.27]	2.9 [2.7]	2.6 [2.4]		0.07 [0.20]	0.14 [0.15]	*Panuwet, P.* et al., *2009*
Spain (Valencia)[c]	125	2010	< LOD [0.47]	5.2 [3.3]		0.93 [0.96]	3.4 [3.36]		< LOD	< LOD	*Roca, M.* et al., *2014*
Germany	396	2001-2002							0.29 [0.31]	< LOD	*Becker, K.* et al., *2006*

[a] < LOD data below limit of detection.
[b] Geometric mean into [ ].
[c] Data in ng/g creatinine

must reflect recent inputs in the studied area. This compound has been found in North Italy as one of the most abundant pesticide (Ghini et al., 2004).

In the case of IMPY (diazinon) and CMHC (coumaphos), the samples examined in the present cohort have median values below the limit of detection which are similar with those observed in previous studies with children (Arcury et al., 2007; Lu et al., 2008; Panuwet et al., 2009). These results are consistent with the ban of these pesticides by the European Union. However, in other studies, e.g. the Valencian cohort (Roca et al., 2014), the IMPY metabolite was dominant, 5.2 ng/mL.

MDA (malathion) is also present at low median concentrations, < LOD, which is a distinct feature comparing from studies in USA or Thailand (Barr et al., 2005; Arcury et al., 2007; Lu et al., 2008; Panuwet et al., 2009). The low contents in the Trieste cohort are in contrast with the occurrence of this OP in some Italian rivers (Montuori et al., 2015).

A statistically significant difference between parents' educational level and children DEAMPY metabolite concentrations has been observed in the present cohort (Table 5), involving higher levels in the group of higher education. Previous studies on general population of women have found similar significant differences with OP pesticide exposure. Thus, women with lower/middle studies were those showing lower concentrations of TCPY in New York (Berkowitz et al., 2003). Similar patterns were observed in women from Cincinnati (Yolton et al., 2013) and Canada (Sokoloff et al., 2016), where women with higher education presented higher urinary concentrations of OP metabolites, dialkylphosphates in these cases. This positive association with education could reflect distinct dietary habits related with cultural differences and higher consumption of products treated with OPs.

4.2. Pyrethroids

Comparing with previous studies, children from Trieste show the highest concentrations of the 3-BPA metabolite, 0.56 ng/mL (Becker et al., 2006; Arcury et al., 2007; Panuwet et al., 2009; Roca et al., 2014). In contrast, the median concentration of 4-F-3-BPA in the studied cohort, < LOD, is similar to those found in previous studies, < LOD-0.14 ng/mL. Some pyrethroids have authorized use in Italy for beekeeping activities (Perugini et al., 2018). They have also been found in wastewater (Rousis et al., 2016).

Although PYR have been found in fish (Corcellas et al., 2015; Muir et al., 1994) no statistically significant associations have been found between PYR or OP concentrations and weekly fish consumption. These results are in agreement with previous studies that did not observe any association with PYR exposure and fish consumption in Rome (Fortes et al., 2013), and in disagreement with a study in a Norwegian cohort (Cequier et al., 2017) that found a positive association with one non-specific OP metabolite. In any case, the EDI calculations from the present cohort shows that children's exposure to OPs pesticides and PYR were within safe levels.

5. Conclusions

DEAMPY was the metabolite found at higher concentration in the urine of Italian children belonging to the NAC-II -PHIME cohort (median 4.5 ng/mL SG adjusted). This metabolite of pirimiphos was followed by PNP and TCPY, the metabolites of parathion and chlorpyrifos, respectively. In contrast, the concentrations of TCPY were low when compared to other distributions of OP metabolites in children. These compositional differences make the Trieste cohort very distinct in terms of OP pesticide composition from other cohorts of children described in previous studies, showing high influence of pirimiphos. Another specific feature of this cohort is the high abundance of 3-BPA, a general marker of PYR pesticides.

A positive association between higher paternal educational levels and higher child urinary concentrations of OPs and PYR metabolites was observed. This difference could reflect distinct dietary habits, with regard to fish consumption, depending on paternal and maternal education. Dietary assessment through a three-day diet diary is ongoing in this cohort, and this will eventually allow one to establish significant association between OP and PYR metabolites and intake of specific food items, e.g. fruits, vegetables, dairy products, further to fish. The concentrations of these OP and PYR pesticides are not related to fish consumption, and estimated daily intakes of all studied metabolites were found within safe levels. However, further investigation is needed to determine the urinary excretion factor in children to being able to evaluate EDIs.

Declaration of interests

The paper reflects only the authors' views, and the European Union is not liable for any use that may be made of the information. The authors declare they have no competing interests.

Acknowledgements

Authors are grateful to the women who participated in our study as well as the people who helped in the recruitment in the different countries. This work was supported by the EU through its Sixth Framework Programme for RTD project PHIME (contract no FOOD-CT-2006- 016253), by GRANT LIFE 2012 ENV/GR/001040 "Crome Life - Cross Mediterranean Environment and Health Network", by European Union projects HEALS (FP7-ENV-2013- 603946) and Neurosome (H2020-MSCA-ITN-2017 SEP-210411486), by RC 12/12 of Institute for Maternal and Child Health -IRCCS "Burlo Garofolo" – Trieste – Italy funded by the Italian Ministry of Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.05.039.

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MATERIAL SUPLEMENTARI

Urinary metabolites of organophosphate and pyrethroid pesticides in children form an Italian cohort (PHIME, Trieste).

Taula de continguts:

Figure S1. Sociodemographic plots of GM values and 95% CI (ng/mL SG adjusted) of metabolites in children. Acronyms of the abscissas refer to the metabolite names of Table 1.

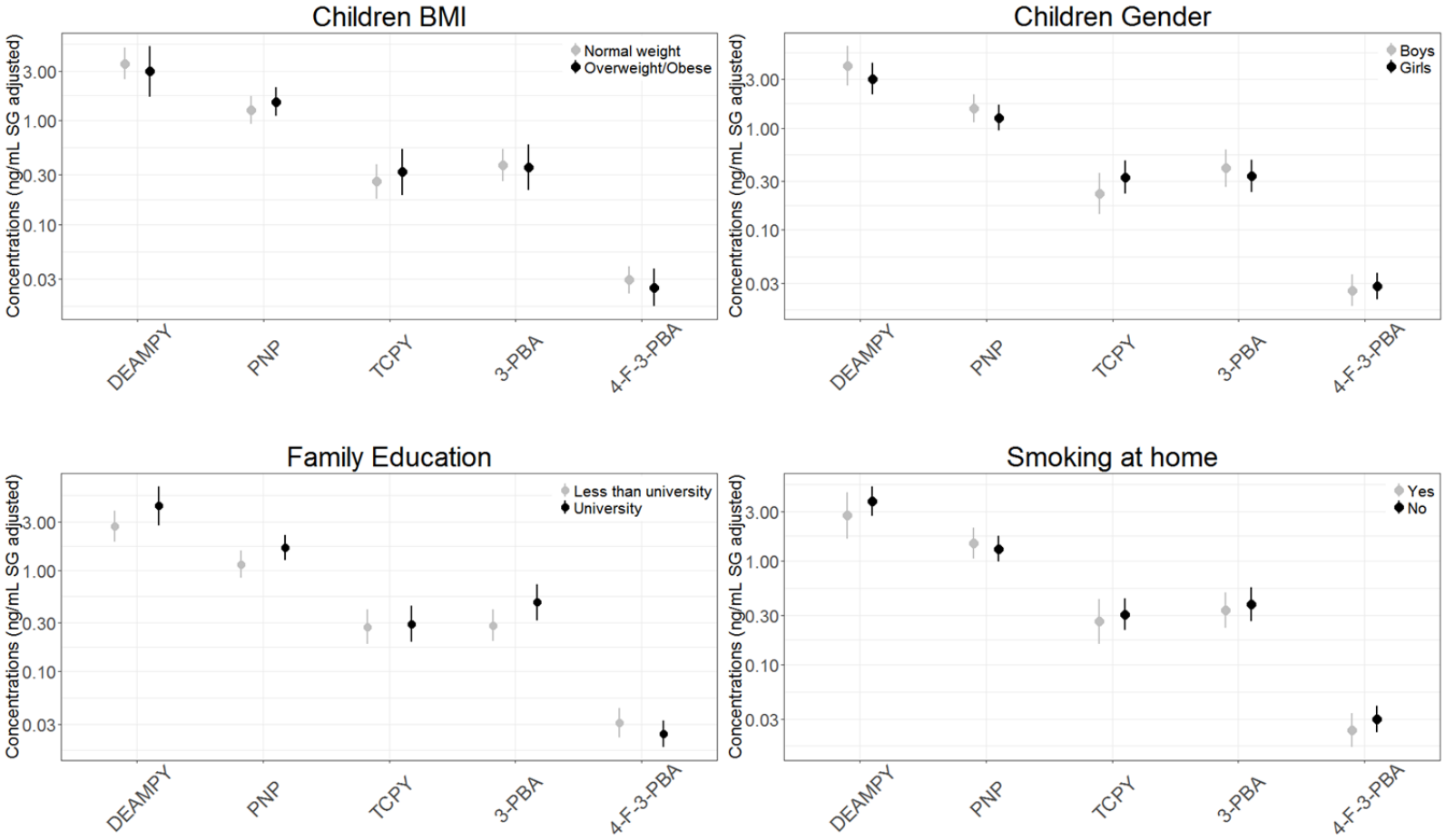

Table S1. β coefficients and *p*-values (in brackets) resulting from the regression models showing effects of fish consumption on organophosphate and pyrethroid pesticides concentrations.

			Organophosphate Pesticides			Pyrethroids Pesticides	
	Servings[1]	**n**	**DEAMPY**	**PNP**	**TCPY**	**3-PBA**	**4-F-3-PBA**
Fresh fish	≥ 1x/wk[2]	98	0.40	0.081	0.059	-0.043	0.32
	< 1x/wk	72	[0.26]	[0.77]	[0.87]	[0.91]	[0.30]
Shellfish	≥ 1x/wk	161	-0.28	0.77	1.1	-0.15	0.78
	< 1x/wk	9	[0.74]	[0.24]	[0.21]	[0.87]	[0.29]
Clams	≥ 1x/wk	158	-0.36	-0.27	0.34	0.0081	-0.21
	< 1x/wk	12	[0.61]	[0.62]	[0.64]	[0.99]	[0.73]
Canned fish (in oil)	≥ 1x/wk	70	-0.050	-0.25	0.074	0.34	-0.12
	< 1x/wk	106	[0.88]	[0.34]	[0.83]	[0.33]	[0.68]

Models adjusted by children gender and BMI and family education

[1]Servings/week 150 g is considered 1 serving; [2]1x/wk: once a week.

ARTICLE 6

Mother/child organophosphate and pyrethroids distributions

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Enviat a: Environmental International (en revisió)

Mother/child organophosphate and pyrethroid distributions

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Keywords: Organophosphorus pesticides, Pyrethroids, Human Biomonitoring, Children, Women, Child-mother pairs.

Abstract

The present study reports one of the few cases in which organophosphate (OP) and pyrethroid (PYR) pesticide human exposure is evaluated in family contexts by the analysis of mother/child pair samples. Urinary concentrations of 6 organic metabolites of organophosphates and 2 pyrethroids were measured in mothers and their 7-to 8-year-old children (n = 168) in a general population from the central area of Slovenia. The results were adjusted for specific gravity and creatinine.

The most abundant OP metabolite in children was 4-nitrophenol (PNP) (median 0.7 ng/ml) and in mothers (0.45 ng/ml), representing parathion exposure. 3-Phenoxibenzoic acid (3-PBA) (0.26 ng/ml), the general metabolite of pyrethroids, and 3,5,6-trichloro-2-pyridinol (TCPY) (0.16 ng/ml; chlorpyriphos) were the second most abundant compounds in children and mothers, respectively. The geometric mean specific gravity adjusted concentrations of OPs and PYRs were statistically significantly higher in children than in their mothers (between 3% and 24% higher), with the exception of TCPY (26% lower). All OP and PYR metabolites found in higher concentration in children showed significant positive correlations with the metabolite concentrations found in the mothers ($p<0.05$ and 0.01), involving the fact that higher maternal concentrations were associated with higher children levels.

These differential mother-children distributions and significant correlations were observed for the 2 types of pesticides studied, OPs and PYRs, which have different chemical properties. This agreement is consistent with the incorporation of the pesticides because of the general activities developed in the family context, instead of pesticide-dependent specific inputs.

Comparison of the estimated daily intakes with the acceptable daily intakes of all detected metabolites revealed no significant risk of adverse health effects from exposure to these pesticides.

Introduction

Organophosphate (OP) and pyrethroid (PYR) pesticides are used in agriculture, gardening, domestic and veterinary applications. They substituted the organochlorine insecticides because of their higher lability to environmental degradation. Their extensive use has involved the occurrence of residues of these compounds in dietary products, water, outdoor and indoor air and house dust (Mercier et al., 2011, Banerjee et al., 2012; Coscollà et al., 2017; Gibbs et al., 2017; Glorennec et al., 2017; Sousa et al., 2018; Tang et al., 2018; van den Dries et al., 2018), and human exposure through diet (Tsatsakis et al., 2003, Fortes et al., 2013; Ciscato et al., 2014, Lewis et al., 2015, Quijano et al. 2016), dermal contact or inhalation (Becker et al., 2006; McKone et al., 2007).

Adult exposure to OP pesticides has been associated with cancer incidence (Engel et al., 2017), deleterious impacts on the reproductive and endocrine systems (Ram, 2017) or diabetes (Starling et al., 2014). PYR pesticides have lower toxicity to mammals than OPs (Naharashi et al., 2007). However, in adults, exposure to these compounds has been found to be significantly related to diabetes (Park et al., 2019), effects on the endocrine system (Santos et al., 2019) and depleted pulmonary function (Kim et al., 2019).

The finding of OPs and PYRs in amniotic fluid and meconium (Berton et al., 2014, Bradman et al., 2003) suggests that foetuses are already exposed to these chemicals. Children are particularly sensitive to environmental toxicants due to their developmental immaturity, particularly the brain and nervous system, and their lower capacity to eliminate xenobiotic chemicals compared to adults (Rice and Barone, 2000; Landrigan et al., 2004; Bjørling-Poulsen et al., 2008; Grandjean and Landrigan, 2014; Slotkin et al., 2019). Prenatal exposure to OP has been related to neurodevelopmental problems (González-Alzaga et al., 2014), low birth weight (Rauh et al., 2012), increased child blood pressure (Harari et al., 2010), short gestation time (Eskenazi, 2004), respiratory difficulties (Reardon et al., 2009; Raanan et al., 2016; Ye et al., 2017), obesity

and diabetes (Debost-Legrand et al, 2016; Slotkin, 2011). Postnatal exposure to these compounds has also been reported deleterious for neurodevelopment, which include mainly deficits in working memory, low attention or motor speed (Ruckart et al., 2004; Rohlman et al., 2005). Exposure to PYR residues has been significantly associated with increases of autism spectrum disorder (von Ehrenstein et al., 2019) and respiratory outcomes (Reardon et al., 2009).

A meta-analysis study on the daily intake and risk assessment of OPs in farmers, general population, children and pregnant women observed differential exposures depending on occupation and age (Katskikantami et al., 2019). These antecedents suggest that the overall family exposure to these compounds may result in uneven impacts in children and adults.

After entry into the body, OPs and PYRs are mostly metabolised by cytochrome P450 enzymes and the hydrolysis products excreted through the urine (Barr, 2008). The hydroxylated organic moieties generated from OP or PYR pesticides (Chambers and Russell, 1995; Mikata et al., 2011) allow for the identification of the precursor compound. The half-lives of most OP and PYR pesticides and their metabolites are in the order of a few hours to some months (Hoffman et al., 2006; Li et al., 2019b). Repeated urine collection and analysis provide information on average exposure over long time periods (Li et al., 2019a). However, single-spot urine analyses can be used for comparative assessment of short-term environmental or dietary exposure (Bradman et al., 2012) between individuals when the samples are collected simultaneously.

In order to get further insight into family exposure to these pesticides and to assess differential impacts in mothers and their 7-to 8-year-old children, exposure to OP and PYR pesticides from 168 mother-children pairs of Ljubljana (Slovenia) was studied by analysis of urine samples collected simultaneously. There were 6 specific urinary OP metabolites, 2-diethylamino-6-methyl pyrimidin-4-ol (DEAMPY), 2-isopropyl-6-methyl-4-pyrimidol (IMPY), malathion dicarboxylic acid (MDA), 4-nitrophenol (PNP), 3-chloro-4methyl-7-

hydroxicoumarin (CMHC) and 3,5,6-trichloro-2-pyridinol (TCPY), representing the organic moieties of pirimiphos, diazinon, malathion, parathion, coumaphos and chlorpyrifos (Supplementary Table S1), that were analysed. The analysis of these metabolites provided specific knowledge on the precursor OP pesticides and, therefore, direct assessment of the exposure of this population, independently of regulations. This information is more elusive when analysing the dialkylphosphate moieties of these compounds. Also measured were 1 specific PYR metabolite, 4-fluoro-3-phenoxybenzoic acid (4-F-3-BPA), representing cyfluthrin, and 3-phenoxybenxoic acid (3-PBA), a metabolite of several PYRs (Supplementary Table S1), were also measured in the urine samples.

Irrespectively of approved regulations, the metabolites selected for analysis were those observed in the urine samples of recent studies (Roca et al., 2014; Gari et al., 2018; Li and Kannan, 2018; Bravo et al., 2019). The incidence of the socio-demographic and morphological characteristics of mothers and children in the observed concentrations have been evaluated. These concentrations were used to calculate estimated daily intakes and to evaluate potential health risks. The present study is one of the few considering simultaneous OP and PYR pesticide exposure in both mothers and children and the first using the organic OP moieties in this context.

Methods

Population and study design

Mother-child pairs were recruited between 2008 and 2009 at the Maternity Hospital of Ljubljana as part of the EU-funded project PHIME (6th FP, Public health impact of long-term, low-level mixed element exposure in susceptible population strata), devoted to identifying associations between low-level exposure to trace elements and critical health effects in susceptible population

groups. Pregnant women eligible for recruitment were permanent residents in the study area (Ljubljana and surroundings–up to 50 km), older than 18 years, had no history of drug abuse, lacked serious health problems or pregnancy complications, and had no twin gestation (Valent et al., 2013). The mother-child pairs were followed up in 2016 as part of the LIFE+ project CROME (Cross-Mediterranean Environment and Health Network). At that time, urine samples were collected from the mothers and their 7-to 8-year-old children at appointed visits at the Paediatric Clinic of the University Medical Centre of Ljubljana. There were 178 pairs resampled out of the 590 pairs recruited in 2008-09. The research protocols of both studies were approved by the National Ethics Committee of the Republic of Slovenia.

Sample preparation and instrumental analysis

The mother-child pairs provided spot urine samples (~ 50 ml). Before analysis, samples were divided intro aliquots and stored at -80°C (Stajnko et al., 2019). The analytical work was performed at the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain.

The procedures of sample preparation and analysis have been described elsewhere (Gari et al, 2018). Briefly, centrifuged and filtered urine samples were introduced into 10-ml centrifuge tubes together with a mixture of isotopically labelled internal standards, where they were hydrolysed with β-glucuronidase. The hydrolysed mixtures were cleaned up using solid-phase extraction. The cartridges were preconditioned with a mixture of MeOH:acetone followed by a solution of acetic acid 1% in H_2O. The OP and PYR metabolites were eluted with a mixture of (1:3) MeOH:acetone. The collected extracts were reduced to near dryness with a gentle N_2 stream and transferred to chromatographic vials with (1:3) MeOH:H_2O. Identification and quantification of the OP and PYR metabolites were carried out using an Ultra-Performance Liquid

Chromatography (UPLC Acquity H-Class, Waters, Milford, MA, USA) equipped with an electrospray ionisation interface. The chromatographic separation was performed on a Betasil C_{18} column.

Synthetic urine was used for blanks, quality control materials and calibration curves. Accuracies were assessed at 2 levels; low and high and calibration curves were prepared by adding 25 µl of standard solutions at concentrations ranging between 2.5 and 800 ppb into synthetic urine. Quantification was performed by isotopically-labelled internal standards (Garí et al., 2018). This methodology has been externally checked out by participation in rounds of the German External Quality Assessment Scheme (G-Equas) since 2016, and includes the organophosphate metabolites PNP and TCPY and the pyrethroid metabolite 3-PBA.

Specific gravity (SG) and creatinine were measured in the urine samples. The SGs ranged from 1.002 to 1.035 g/ml in children and from 1.002 to 1.032 g/ml in women; means were 1.017 and 1.012 g/ml, respectively. The SG corrected concentrations were obtained by applying the following equation: $[OPs\ or\ PYR(ppb)]_{SG} = [OPs\ or\ PYR(ppb)] * ((\bar{x}_{SG} - 1)/(SG(g/mL) - 1)$ (Boeniger et al., 1993), where [OPs or PYR] is the concentration of OPs or PYRs; $\bar{x}_{SG}$ is the specific gravity mean and SG the specific gravity of each participant. Creatinine concentrations ranged between 0.3 and 34.8 mmol/L.

Calculation of OP and PYR daily intakes

Pesticide regulation committees such as the European Commission report the maximum pesticide acceptable doses as acceptable daily intakes (ADI) and reference doses (RfDs) expressed as µg/(kg body weight day). Thus, OP and PYR estimated daily intake (EDI) were calculated from the analysed pesticides using the molar levels of the urinary metabolites, according to the following mathematical model (Katsikantami et al., 2019): $EDI\left(\frac{\frac{\mu g}{kg\ bw}}{d}\right) = \frac{C_U\left(\frac{\mu mol}{L}\right) * V_U(L) * MW_P\left(\frac{g}{mol}\right)}{F_{UE} * BW\ (kg)}$, where

EDI is the Estimated Daily Intake; C_U the molar concentration of the metabolite; V_U the total urinary volume excreted within 24 h; MW_P the molecular weight of the parent compound; F_{UE} the urinary excretion factor of the parent compound and BW the body weight.

The data used for these calculations are shown in the Supplementary Table S2. The 24-h urine volumes were estimated as 1.6 L for mothers and 0.82 L for children (Cequier et al., 2017). The molecular weight of the PYRs having 3-PBA as main metabolite was estimated from the mean of the most common pyrethroids (permethrin, deltamethrin, fenvalerate and cypermethrin). Urinary excretion factors (F_{UE}) were obtained for all metabolites (Morgan et al., 1977, Wollen et al., 1992, Leng et al., 1997, Bouchard et al., 2003, Payne-Sturges et al., 2009, Sams and Jones, 2012 and Ratelle et al., 2015) except DEAMPY. Hence, 2 approaches were chosen for the calculations, 1 using the reported F_{UE} and the other assuming a minimum of 5% and a maximum of 100% excretion.

Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). Statistics was focused on the metabolites found above limit of detection in more than 30% of the samples: DEAMPY, PNP, TCPY, 3-PBA and 4-F-3-PBA (16% for 4-F-3-PBA in mothers). There was 1/2 of the limits of detection assigned to non-detected values (EPA, 2006).

Geometric means (GM) and 95% confidence intervals (CI), as well as the median were used for descriptive analysis. Differences between children and mothers were evaluated both independently and paired, using the Mann-Whitney and Spearman correlation test, respectively. Differences of OP and PYR pesticides levels by socio-demographic factors have been assessed using the Mann-Whitney U-test. Creatinine levels fluctuate more than urine's specific gravity between age and gender (Suwazono, 2005). In this study SG, adjusted

levels have been used for data analysis while adjusted levels are provided for comparison purposes.

Results and discussion

Socio-demographic characteristics

A description of the 168 families included in the study is summarised in Supplementary Table S3. The information was obtained through questionnaires administered during pregnancy, at delivery and when children were 7-8 years old. The average age of the participating women was 38.8 years, with an overall age range between 30 and 51 years. According to BMI, 33% of the women were overweight or obese and 67% had normal weight.

The highest family educational level, either from the mother or the father, was used. Due to unequal distribution, the 5 categories of Supplementary Table S3 were grouped as university studies and higher (n=101, 60%) or less than university studies (n=67, 40%) for statistical analysis.

Concerning children, 51% were boys and 49% girls; their average weights and heights were 28.7 kg and 133.7 cm, respectively (Supplementary Table S3). The gender differences of weight and height were statistically significant ($p<0.001$; Mann-Whitney U Test). Almost all children had normal BMI (79%). They were 13 and 11 who were overweight or obese, respectively, and only 2 were underweight (WHO, 2018).

Concentrations of urinary OP and PYR metabolites

Descriptive statistics for the measured urinary concentrations of the analysed metabolites are reported in Table 1. The compound detection frequencies ranged from not detected to 98% and 92% detection in children and mothers, respectively, PNP and TCPY being the most frequently found metabolites in both cases and 92% and 84% for PNP and TCPY in mothers, respectively).

Table 1. Specific gravity (ng/mL) and creatinine (ng/g creatinine) adjusted and non-adjusted urinary levels (ng/mL) of OP and PYR metabolites.

			Specific gravity adjusted results				Creatinine adjusted results				Non-adjusted results			
Metabolite	**LD[1] (ng/mL)**	**DF[2] (%)**	**GM**	**(CI)[3]**	**Median**	**Range**	**GM**	**(CI)**	**Median**	**Range**	**GM**	**(CI)**	**Median**	**Range**
Children n=164														
DEAMPY	0.017	69	0.23	(0.16-0.31)	0.32	nd-23	0.35	(0.26-0.49)	0.44	nd-62	0.19	(0.13-0.27)	0.30	nd-22
IMPY	0.014	30	0.019	(0.015-0.023)	<LD	nd-0.72	0.030	(0.024-0.038)	<LD	nd-1.1	0.015	(0.013-0.019)	<LD	nd-1.053
MDA	0.069	9	<LD		<LD	nd-4.3	<LD		<LD	nd-13	<LD		<LD	nd-2.1
PNP	0.017	98	0.85	(0.71-1.0)	0.93	nd-11	1.3	(1.1-1.6)	1.4	nd-20	0.70	(0.57-0.86)	0.84	nd-23
TCPY	0.02	88	0.083	(0.064-0.11)	0.054	nd-12	0.13	(0.099-0.18)	0.086	nd-25	0.069	(0.057-0.082)	0.057	nd-2.8
CMHC	0.026	0	<LD		<LD	-	<LD		<LD	-	<LD		<LD	-
3-PBA	0.018	80	0.31	(0.23-0.41)	0.52	nd-29	0.48	(0.35-0.66)	0.84	nd-59	0.26	(0.19-0.34)	0.40	nd-12
4F-3-PBA	0.019	29	0.025	(0.019-0.032)	<LD	nd-2.1	0.040	(0.030-0.053)	<LD	nd-4.0	0.021	(0.017-0.025)	<LD	nd-0.53
ΣOP[4]	-	85	1.9	(1.7-2.2)	2.0	0.12-24	3.0	(2.6-3.6)	3.2	0.074-74	1.6	(1.4-1.9)	1.7	0.10-23
ΣPYR[5]	-	55	0.42	(0.33-0.54)	0.55	nd-30	0.66	(0.50-0.86)	0.89	0.012-59	0.35	(0.27-0.44)	0.52	0.019-12
Mother n=168														
DEAMPY	0.017	35	0.07	(0.045-0.095)	<LD	(nd-46)	0.12	(0.080-0.17)	<LD	(nd-65)	0.05	(0.035-0.077)	<LD	(nd-73)
IMPY	0.014	7	<LD		<LD	(nd-0.83)	<LD		<LD	(nd-1.8)	<LD		<LD	(nd-0.41)
MDA	0.069	3	<LD		<LD	(nd-3.3)	<LD		<LD	(nd-7.3)	<LD		<LD	(nd-0.58)
PNP	0.017	92	0.57	(0.45-0.71)	0.73	(nd-7.3)	1.0	(0.80-1.3)	1.1	(nd-31)	0.45	(0.35-0.58)	0.61	(nd-9.6)
TCPY	0.02	84	0.20	(0.15-0.27)	0.26	(nd-9.3)	0.36	(0.26-0.48)	0.446	(nd-19)	0.161	(0.13-0.21)	0.181	(nd-4.5)
CMHC	0.026	1	<LD		<LD	(nd-0.075)	<LD		<LD	(nd-0.29)	<LD		<LD	(nd-0.11)
3-PBA	0.018	67	0.16	(0.12-0.22)	0.40	(nd-18)	0.28	(0.20-0.39)	0.62	(nd-34)	0.13	(0.094-0.17)	0.24	(nd-12)
4F-3-PBA	0.019	16	<LD		<LD	(nd-1.7)	<LD		<LD	(nd-4.9)	<LD		<LD	(nd-0.73)
ΣOP[4]	-	70	1.6	(1.3-1.9)	1.7	(0.012-50)	2.8	(2.4-3.4)	3.1	(0.023-70)	1.3	(1.0-1.6)	1.3	(0.026-74)
ΣPYR[5]	-	42	0.23	(0.17-0.30)	0.46	(0.0084-18)	0.40	(0.31-0.53)	0.67	(nd-35)	0.18	(0.14-0.23)	0.26	(nd-12)

[1]LD: Limit of detection; [2]DF: Detection frequencies; [3]GM (CI): Geometric mean and 96% confidential interval;
[4]ΣOP = [DEAMPY] + [PNP] + [TCPY]; [5]ΣPYR = [3-PBA] + [4-F-3-PBA]

The sum of the most abundant organophosphate pesticide metabolites, PNP, TCPY and DEAMPY, ranged between 0.074 and 74 µg/g creatinine in children, with a median of 3.2 µg/g creatinine, and between 0.023 and 70 µg/g creatinine in the mothers, with a median of 3.1 µg/g creatinine. Total pyrethroids, 3-PBA and 4F-3-PBA, was substantially lower in both groups, with ranges of 0.012-59 µg/g creatinine in children, with a median of 0.89 µg/g creatinine, and nd-35 µg/g creatinine, in mothers, with a median of 0.67 µg/g creatinine (Table 1).

The most abundant compound in children and mothers was PNP, the parathion metabolite, with medians of 1.4 and 1.1 µg/g creatinine in children and mothers, respectively. The next most abundant compound in children was the general metabolite of pyrethroids 3-PBA, with a median of 0.84 µg/g creatinine. In mothers, the next most abundant was TCPY, the chlorpyriphos metabolite, with a median of 0.45 µg/g creatinine. The dominance of parathion is unexpected because the use of this compound was banned in several countries, including the European Commission, since 2004. However, despite this ban, it has been found in the human urine of diverse populations, such as Queensland (Australia), and, in view of the relatively short environmental half-life of this pesticide, its occurrence was attributed to current use of previous stocks (Li et al., 2019b). In France, the presence of this compound and other banned pesticides has also been attributed to use of previously stored plant protection products or fruits or vegetables from African countries (Boucaud-Maitre et al., 2019).

Comparison with other studies

The present cohort shows highest SG adjusted concentrations of 3-PBA in children (0.4 ng/ml) when compared with children's measurement in Germany, France, the US, Spain and Thailand (0.02-0.29 ng/ml; Table 2). In contrast, the most abundant OP metabolite, 0.84 ng/ml, does not involve a high level when compared with concentrations described in studies from the USA, Spain and

Table 2. Comparison of 50th percentile data (ng/mL) in children's urine samples with other similar studies

	n	Year	DEAMPY	IMPY	MDA	PNP	TCPY	CMHC	3-PBA	4-F-3-PBA	Study
Ljubljana (Slovenia)	164	2014-2015	0.30 (0.009-1.3)[1] [0.19][3] (0.13-0.27)	<LD[2] [0.015] (0.013-0.019)	<LD <LD	0.84 (0.35-1.7) [0.70] (0.57-0.86)	0.057 (0.031-0.17) [0.069] (0.057-0.082)	<LD <LD	0.4 (0.14-0.81) [0.26] (0.19-0.34)	<LD [0.021] (0.017-0.025)	*Present study*
Brittany (France)	245	2009-2012							0.02 (0.05-0.08)[b]	<LD	*Glonnerec et al., 2017*
US (NHANES)	481	1999-2000			0.49 (0.38-0.7)[c]		2.7 (1.8-4.2)[c] [2.88] (2.13-3.88)				*Barr et al., 2005*
North Carolina (US)	60	2004	0.14	0.49 (3.94)[d] [0.56]	0.21 (6.87)[d]	1.55 (6.32)[d] [1.00]	2.47 (16.91)[d] [1.92]	0.14	0.07		*Arcury et al., 2007*
Seattle (US)	23	2003-2004	<LD	<LD	1.6 (0-3.6)		3.7 (1.5-7.5)	<LD			*Lu et al., 2008*
Thailand	207	-	0.14 [0.16] (0.25-6.31)[e]	<LD	0.21 [0.27] (0.32-2.13)[e]	2.87 [2.68] (0.28-19.5)[e]	2.64 [2.35] (0.20-34.5)[e]		0.07 [0.20] (0.03-74.0)[e]	0.14 [0.15] (1.37-2.18)[e]	*Panuwet et al., 2009*
Valencia (Spain)[a]	125	2010	<LD [0.47] (0.35-0.64)	5.16 (<LD-11.70) [3.31] (2.52-4.35)		0.93 (<LD-1.61) [0.96] (0.81-1.13)	3.4 (1.91-6.16) [3.36] (2.74-4.10)		<LD	<LD	*Roca et al., 2014*
Germany	396	2001-2002							0.29 (2.35)[d] [0.31] (0.28-0.34)	<LD	*Becker et al., 2006*

[1]Interquartilic range (p25-p75) and 95% confidencial interval for 50th percentile and geometric mean, respectively, in parentheses; [2]<LD data below limit of detection; [3]Geometric mean into [ ]

[a]Data in ng/g creatinine; [b]p75-p90; [c]95% confidence interval; [d]95th percentile; [e]Range (min-max)

Table 3. Comparison of 50th percentile data (ng/mL) in mother's urine samples with other similar studies

	n	Population	Year	DEAMPY	IMPY	MDA	PNP	TCPY	3-PBA	4-F-3-PBA	Study
Ljubljana (Slovenia)	168	Women	2014-2015	<LD [0.052][3] (0.035-0.077)[1]	<LD[2] <LD	<LD <LD	0.61 (0.23-1.4) [0.45] (0.35-0.58)	0.18 (0.060-0.52) [0.16] (0.13-0.21)	0.24 (0.009-0.49) [0.13] (0.094-0.17)	<LD <LD	*Present study*
Greece	40	General	2010-2014		0.3 [0.3]	0.4 [0.4]	1.6 [1.6]	6.1 [5.5]	0.5 [0.6]	0.01 [0.01]	*Jing Li et al., 2018*
Queensland (Australia)	200	Women	2012-2013		[0.34]	[0.81]	[1.3]	[17]	[0.86]	<LD	*Heffernan et al., 2016*
US (NHANES)	355	Women	2009-2010		<LD	<LD	0.5 (0.1-1.1)	1.0 (0.5-2.0)			*Lewis et al., 2015*
Puerto Rico (US)	152	Women[4]	2010-2012		<LD	<LD	0.5 (0.3-1.1)	0.5 (0.2-0.9)			*Lewis et al., 2015*
Altanta (US)[a]	55	General	2012	1.0 (0.17-2.82)[b]	0.75 (0.19-2.01)[b]	1.18 (0.39-5.90)[b]	1.4 (0.038-8.16)[b]	1.87 (0.13-6.05)[b]	1.52 (0.066-8.50)[b]	0.84 (0.091-2.25)[b]	*Davis et al., 2013*
Rotterdam (Netherlands)	100	Women[4]	2002-2006					1.2 (0.6-2.4)	1.2 (0.5-2.5)		*Ye et al., 2008*
China	86	General	2010-2014		0.2 [0.2]	0.6 [0.5]	5.0 [5.0]	3.4 [3.7]	0.6 [0.7]	<LD [0.02]	*Jing Li et al., 2018*
Vietnam	22	General	2010-2014		0.2 [0.2]	0.4 [0.5]	2.6 [2.6]	9.3 [9.3]	0.1 [0.05]	<LD <LD	*Jing Li et al., 2018*

[1] Interquartilic range (p25-p75) and 95% confidencial interval for 50th percentile and geometric mean, respectively, in parentheses; [2]<LD data below limit of detection; [3]Geometric mean into [ ]; [4]Pregnant women.

[a]Average results; bRange (min-max)

Thailand, at 0.93-2.87 ng/ml (Table 2). The same is the case for TCPY, IMPY and MDA, whose median concentrations in children of the Slovenian cohort are low at 0.057 ng/ml, <LD and <LD, respectively, when compared with those of previous countries at 2.47-3.7 ng/ml, <LD-5.16 ng/ml and 0.21-1.6 ng/ml, respectively. Finally, DEAMPY, the pirimiphos metabolite, the second most abundant OP metabolite in the present cohort at 0.30 ng/ml, shows the highest reported value in comparison with previous studies in the USA, Thailand and Spain, at <LD-0.14 ng/ml (Table 2).

The comparison with the OP and PYR metabolite concentrations of the Slovenian mothers participating in the study has been performed with another series of reference cohorts (Table 3) because children and mothers are rarely considered together in studies involving these pesticides. As shown in Table 3, the concentrations of OP and PYR metabolites in the Slovenian mothers generally range among the lowest described in previous studies in Greece, Australia, the USA, the Netherlands, China and Vietnam. Only in a few cases, such as 3-PBA in Vietnam (0.1 ng/ml) and PNP in some US studies, NHANES and Puerto Rico at 0.5 ng/ml, are the concentrations lower than those of the present study (Table 3).

Differences between mother and child metabolite concentrations

In all cases except TCPY, the geometric mean SG adjusted concentrations of OPs and PYRs were higher in the 7-year-old children than in their mothers (n = 163; Figure 1), and the differences were statistically significant for DEAMPY, IMPY, TCPY and 3-PBA. Studies with DAPs have found higher concentrations in mothers than in children, e.g. in the US National Health and Nutrition Examination Survey (NHANES, Xue et al., 2014) or 6 European mother-child cohorts (Haug et al., 2018). However, in other cases the differences did not follow a uniform trend (Cequier et al., 2017), and in others, the observed concentrations were higher in children, e.g. in the Canadian Health Measures

Survey (CHMS, Health Canada, 2010). These discrepant results may reflect the higher rate of metabolic elimination of alkylphosphate moieties than of the organic OP metabolites.

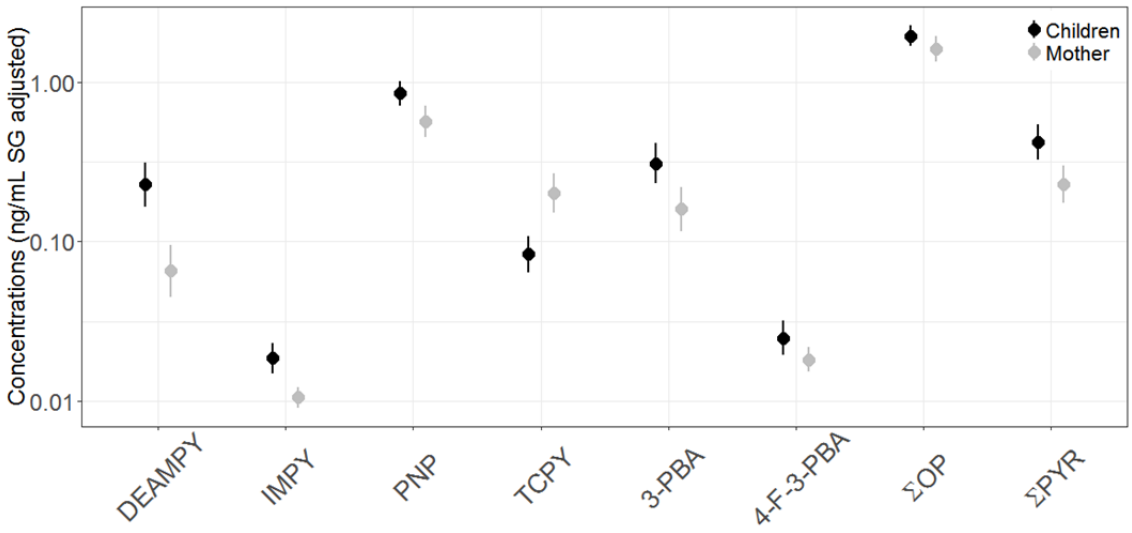

Figure 1. GM levels of OPs and PYR for the mother-child pairs (ng/mL SG adjusted).

All metabolites found in higher concentration in children in the Slovenian cohort, DEAMPY, PNP and 3-BPA (Table 1), together with 4F3PBA, showed significant correlations with the metabolite concentrations found in the mothers ($p<0.01$ and 0.05; Table 4). These correlations were calculated over SG adjusted concentrations, and they were also observed when the concentrations were non-adjusted or creatinine adjusted.

Previous studies on OP pesticides in mothers and children using DAPs only found significant correlations in 1 case (Norway, Cequier et al., 2017), which again may reflect the lower retention of these OP pesticide moieties in comparison to the organic metabolites. The lower retention effect likely overcomes the metabolite concentration differences between mother and children. In this respect, the PYR metabolites analysed in the present paper show the same trends as with the OP organic moieties, indicating a consistent trend for these pesticides of different chemical properties.

All significant correlation coefficients found in the present study were positive, involving the fact that higher maternal concentrations were associated with higher children concentrations. The highest correlation significance ($p<0.01$) was found for PNP, the metabolite in highest concentration in children and mothers (Table 1). These significant correlations are consistent with coherent distributions of OP and PYR metabolites in children and their mothers, reflecting parallel responses to the same inputs, e.g. those involving exposure of the whole family to these pesticides.

Further assessment on the relative accumulation of these pesticide metabolites in mothers and 7-to 8-year-old children can be obtained from compilation of the child/(child + mother) ratios using the observed SG-adjusted urinary concentrations (Table 1). As shown in Table 4, these ratios are higher than 0.5 in all cases (higher concentrations in children than in mothers) except TCPY. This compound is one of the few metabolites that does not show significant correlation between the concentrations in mothers and their children (Table 4). Besides this lack of correlation, TCPY is the only metabolite found in higher concentration in mothers than in children, suggesting that perhaps this pesticide was incorporated outside the common family life activities.

Table 4. Spearman correlations of the urinary SG adjusted concentrations of OPs and PYR and descriptive statistics of the relationship (child/(mother+child)) of the urinary SG adjusted concentrations of OPs and PYR metabolites in mothers and their seven-year old children (n = 162)

	rho	p	AM	(CI)[1]	Median	Range
DEAMPY	0.18	p<0.05	0.64	(0.58-0.69)	0.74	0.0011-1.0
IMPY	-0.0016	0.98	0.58	(0.53-0.62)	0.56	0.018-0.99
PNP	0.24	p<0.01	0.56	(0.52-0.60)	0.61	0.035-1.0
TCPY	0.15	0.065	0.37	(0.32-0.42)	0.24	0.0010-1.0
3PBA	0.16	p<0.05	0.57	(0.52-0.62)	0.59	0.0058-1.0
4F3PBA	0.17	p<0.05	0.54	(0.49-0.58)	0.53	0.021-0.99
OPSum	0.24	p<0.01	-			
PYRSum	0.16	p<0.05	-			

[1]AM (CI): Arithmetic mean and 95% confidence interval

In any case, the higher occurrence of most OP and PYR metabolites in children and the correlation of concentrations in mother and children suggest that the overall family exposure is retained to a higher extent in children than in the mothers, which is consistent with a less developed metabolism in 7-to 8-year-old children than in adults. Alternatively, the higher concentrations of these pesticide metabolites in children may reflect higher rates of food consumption per body weight than in adults (Dewalque et al., 2014, Katsikantami et al., 2016, Zentai et al., 2016).

Thus, the present study reports that 2 distinct chemical types of pesticides show the same trends, involving correlation of concentrations of mothers and children and higher significant levels of pesticide metabolites in children than in mothers. This agreement between pesticides of different chemical composition and sources supports that the observed distribution differences may be related to metabolic differences or feeding patterns between mothers and their children.

Estimated daily intakes (EDIs)

The median OP and PYR EDIs with interquartile range (Q_1-Q_3) for children and mothers are presented in Figure 2. There has been 3 different F_{UE} considered. The ADIs are also indicated. The differences between children's and mother's EDIs are statistically significant ($p<0.001$, Mann-Whitney U Test) for all pesticides except TCPY (Table 5). These differences are consistent with the above reported higher concentrations of these pesticide metabolites in children than in their mothers. A previous study (Katsikantami et al., 2019) calculating estimated daily intakes from biomonitoring studies showed similar distributions; children had greater exposure to organophosphates compared to adults and the EDIs of parathion were higher than those of diazinon.

The F_{UE} values used in Figure 2 agree with those considered in previous studies (Morgan et al., 1977, Wollen et al., 1992, Leng et al., 1997, Bouchard et al., 2003, Payne-Sturges et al., 2009, Sams and Jones, 2012 and Ratelle et al.,

2015). The hypothetical worse-case for F_{UE} has been assigned to 5% excretion. In these conditions, PNP is crossing the ADI threshold in children, and EDI and ADI are very similar in the mothers. However, this is an unusual case and, in general, all EDIs are below the ADI values, indicating that the exposure to OPs and PYRs occurs within safe levels. Previous EDI studies, using the same mathematical model as in the present Slovenian case, generally showed exposures for children and adults within safe levels (Katskikantami et al., 2019, Bravo et al., 2019). Only 1 study from the US (Kissel et al., 2005) showed a high children exposure to parathion and chlorpyrifos. Other studies using similar models for EDI assessment showed values lower than ADIs for all the studied compounds (Ye et al., 2009; Liu et al., 2014; Yang and Yiin, 2018) but very similar EDIs and ADIs for chlorpyrifos in 1 case of pre-schooler children in Austria (Li et al., 2019b). Nevertheless, the recommended chlorpyrifos ADI has been discussed (Mie et al., 2018) and a value under 1 mg/(kg·bw/day) has been proposed, which is lower than the accepted value by regulatory agencies, e.g. EPA and EFSA. These authors also suggest that the current evaluation procedures for pesticides may need to be modified to ensure that the public is not exposed to substances that are deleterious for human health.

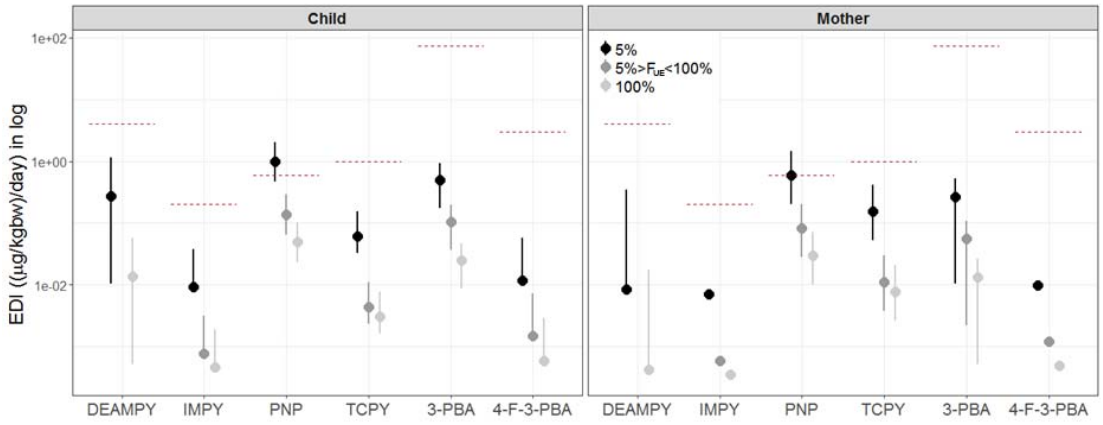

Figure 2. Median estimated daily intakes with interquartile rante (Q_3-Q_1) for children and mothers. In red, acceptable daily intakes.

Table 5. Median estimated daily intakes for children and others and Mann-Whitney U test results.

	Child ((µg/kgbw)/day)			Mother ((µg/kgbw)/day)			p-value
	5%	F_{UE}	100%	5%	F_{UE}	100%	
DEAMPY	0.27	-	0.014	0.0084	-	0.00042	p<0.001
IMPY	0.0092	0.00077	0.00046	0.0071	0.00059	0.00035	p<0.001
PNP	1.00	0.14	0.050	0.59	0.081	0.029	p<0.001
TCPY	0.060	0.0043	0.0030	0.15	0.011	0.0077	p<0.001
3-PBA	0.50	0.10	0.025	0.26	0.055	0.013	p<0.001
4-F-3-PBA	0.012	0.0015	0.00060	0.0097	0.0012	0.00048	p<0.001

Mother-child characateristics and influence on OP and PYR pesticide concentrations

Different maternal and infant characteristics that may influence the urinary OP and PYR pesticide concentrations have been evaluated, as shown in Table 6. The assessed variables have been grouped into 2 categories, and the results are reported as median values and interquartile ranges. The significance of the median differences has been evaluated with the Mann-Whitney U-test. Children show no significant differences with respect to gender, BMI or family educational level for any OP or PYR metabolites. Mothers show no differences on age, education or smoking habits for any metabolite. The lack of association between tobacco smoking and urinary OP or PYR metabolites contrasts with some previous studies, in which statistically significantly higher concentrations of OPs were found among non-smoking women in Valencia (Llop et al., 2017) or the Netherlands (van den Dries et al., 2018) and higher concentrations of 3-PBA were found among smoking woman in the USA (NHANES, Riederer et al., 2008).

Concerning TCPY in the mothers of the present study, there is a significant difference between the normal weight and the overweight/obese group, with the former having statistically significantly higher concentrations. The difference has also been observed for the generic OP metabolites (DAPs) in studies of Valencia (Llop et al., 2017) and Canada (Sokoloff et al., 2016), in

which women with higher BMI were those showing lower concentrations of this OP metabolite.

Table 6. Median urinary concentration differences (ng/mL SG adjusted) of principal metabolites detected by characteristics of study population.

Characteristics	n(%)	DEAMPY		PNP		TCPY		3-PBA	
		P50	IQR[1]	P50	IQR[1]	P50	IQR[1]	P50	IQR[1]
Children	164 (100)								
Gender									
Boys	83 (51)	0.44	0.064-1.5	0.94	0.46-1.6	0.041	0.020-0.23	0.50	0.23-0.95
Girls	80 (49)	0.23	0.029-1.0	0.91	0.42-1.9	0.095	0.028-0.41	0.56	0.086-1.3
BMI[2]									
Normal	127 (80)	0.27	0.038-1.0	0.94	0.39-1.8	0.055	0.023-0.36	0.49	0.066-1.0
Overweight/Obese	32 (20)	0.48	0.10-1.9	0.92	0.48-1.4	0.055	0.022-0.15	0.53	0.38-1.4
Family education									
Below university	66 (40)	0.28	0.051-1.0	1.0	0.41-1.6	0.052	0.027-0.23	0.45	0.17-1.0
University degree	97 (60)	0.37	0.031-1.4	0.85	0.46-1.8	0.058	0.019-0.36	0.56	0.16-1.2
Mothers	167 (100)								
Age									
< 40 years old	106 (63)	0.026	0.012-0.66	0.80	0.35-1.7	0.29	0.080-0.90	0.40	0.018-0.64
≥ 40 years old	61 (37)	0.021	0.0074-0.90	0.67	0.34-1.3	0.17	0.050-0.48	0.40	0.013-0.63
BMI[2]									
Normal	112 (67)	0.021	0.010-0.64	0.80	0.34-1.7	**0.31***	**0.085-0.92**	0.40	0.021-0.64
Overweight/Obese	56 (33)	0.026	0.0092-0.67	0.69	0.34-1.3	**0.18***	**0.029-0.49**	0.39	0.013-0.60
Mother education									
Below university	75 (45)	0.021	0.013-0.65	0.67	0.37-1.3	0.24	0.067-0.82	0.41	0.043-0.64
University degree	93 (55)	0.026	0.0086-0.67	0.80	0.34-1.7	0.30	0.056-0.70	0.38	0.013-0.63
Smoking									
Yes	27 (16)	0.035	0.018-0.58	0.57	0.16-1.6	0.24	0.12-0.49	0.32	0.011-0.77
No	141 (84)	0.021	0.0094-0.67	0.78	0.40-1.6	0.29	0.053-0.80	0.40	0.021-0.63

[1]IQR: Interquartilic range (P25-P75); [2]BMI: Body mass index; **p-valor* <0,05 pel test Mann-Whitney

Strengths and limitations

The present study of OPs and PYR in mother and child pairs uses a robust analytical methodology (Garí et al. 2018). It is one of the few studies analysing 2 types of pesticides in urine of mothers and their children simultaneously, and it is the first with this sampling approach focussing on the OP organic moieties. This approach has allowed us to assess the maternal/infant exposure in the family contexts. A shortcoming of the study is the lack of specific diet information for each family. The use of the same F_{UE} parameter for children and

adults for the calculations of EDIs is also a limitation, since metabolism and excretion of chemical pollutants in children are different than in adults.

Low detection frequencies for some compounds might have introduced some bias into the statistical analysis. In mothers, the detection frequencies of IMPY and 4-F-3-PBA metabolites were below 30%. Statistics was performed for these compounds anyway to be able to compare with the children concentrations of these metabolites, in which they were found above 30% limit of quantification.

Conclusions

PNP is the most abundant OP metabolite in children and mothers, representing parathion exposure. 3-PBA, the general metabolite of pyrethroids, was the second most abundant compound in children, showing higher concentrations than in reports from other countries. In mothers, the next more abundant was TCPY, representing chlorpyriphos.

The geometric mean concentrations of OPs and PYRs were statistically, significantly higher in children than in their mothers, except for TCPY. All OP and PYR metabolites found in higher concentration in children showed significant positive correlations with the metabolite concentrations found in the mothers, involving the fact that higher maternal concentrations were associated with higher children concentrations. These results are consistent with a common family exposure to OPs and PYRs, involving higher accumulation in children than in the mothers which is consistent with a lower capacity of elimination of these pesticides in the former.

These differential mother-children distributions and significant correlations are observed for 2 different types of pesticides, OPs and PYRs, which have different chemical properties. Thus, with the exception of TCPY (chlorpyriphos), the main drivers of the distribution of the studied metabolite pesticides are

determined by the food consumption and life-style of the families, instead of specific inputs related with specific pesticides.

A comparison of EDIs with ADIs for all the detected metabolites revealed no risk of adverse health effects from exposure to these pesticides. Only the hypothetical worst scenario (F_{UE}=5%) showed a possible parathion risk for children, but in general, OP and PYR exposure occurs within safe levels.

Acknowledgements

The authors are grateful to all women who participated in the study, the participating hospital for the data sampling and all the field workers for their enthusiastic efforts. This paper was supported by the European Union project CROME (LIFE12 ENV/GR/001040). Additional funding was received from the European Union projects HEALS (FP7-ENV-2013- 603946), PHIME (FOOD-CT-2006-016253), Neurosome (H2020-MSCA-ITN-2017 SEP-210411486), EDC-MET (H2020-HEALTH/0490-825762) and the Slovenian Research Agency–ARRS (program P1-0143; project NEURODYS–contract no. JZ-9400).

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MATERIAL SUPLEMENTARI

Mother/child organophosphate and pyrethroid distributions.

Taula de continguts:

Table S1. Metabolite's acronyms and principal uses of the pesticides analysed.

Acronym	Analyte	Pesticide	Principal uses	Legal status[1]
DEAMPY	2-diethylamino-6-methylpyrimidin-4-ol	Pirimiphos	All crops, specially fruits and citrus plantations and agricultural facilities	Approved[2]
IMPY	2-isopropyl-6-methyl-4-pyrimidol	Diazinon		Not approved (2007/393)
MDA	Malathion dicarboxylic acid	Malathion		Approved
PNP	4-nitrophenol	Parathion		Not approved (01/520/EC)
TCPY	3,5,6-trichloro-2-pyridinol	Chlorpyriphos		Approved
CMHC	3-chloro-4-methyl-7-hydroxicoumarin	Coumaphos	Farm and domestic animals to control mite	Not approved[3]

[1]European Comission; [2]Pirimiphos-ethyl is not approved (2002/2076); [3]Never notified and authorised in EU.

Table S2. Data used for the calculation of children's and mother's EDI and corresponding ADIs for each metabolite

	MW_M (g/mol)	MW_P (g/mol)	F_{UE}	Study	ADI[1]
DEAMPY	181.23	305.33	-		4
IMPY	152.19	304.35	0.6	*Payne-Sturges, 2009*	0.2
PNP	139.11	291.26	0.36	*Morgan, 1977*	0.6
TCPY	198.43	350.57	0.7	*Payne-Sturges, 2009*	1
3-PBA	214.22	462.92	0.24	*Various authors*[a]	72.5
4F-3-PBA	232.21	434.30	0.4	*Leng, 1997*	3

$MW_{m,}$ Metabolite molecular weight; MW_P, Parent compound molecular weight; F_{UE}, Urinary excretion factor.
[1]ADI: Acceptable Daily Intake (mg/(kg·bw/day)) (European Commission)
[a]Wollen et al., 1992, Sams and Jones, 2012, Ratelle et al., 2015

Table S3. Demographics of study participants

	Participants n (%)
All women	168 (100)
Age (n=167)	38.8±4.0
BMI[a] (n=168)	
Normal weight	112 (67)
Overweight	31 (18)
Obese	25 (15)
Smoking (n=168)	
Yes	27 (16)
No	141 (84)
Family educational level (n=168)	
Apprenticeship	2 (1)
Secondary School	37 (22)
High School	28 (17)
University	70 (42)
Master or PhD	31 (18)
Children	164 (100)
Gender (n=164)	
Boys	83 (51)
Girls	80 (49)
Weight (n=161)	28.7±5.3
Boys (n=83)	29.3±5.4
Girls (n=78)	28.0±5.1
Height (n=159)	133.7±5.8
Boys (n=81)	133.6±6.3
Girls (n=78)	131.7±5.1
BMI[a,b] (n=168)	
Underweight	2 (1)
Normal weight	125 (79)
Overweight	21 (13)
Obese	11 (7)

ARTICLE 7

Organophosphate metabolite concentrations in maternal urine during pregnancy.

Natalia Bravo, Soraya Peralta, Joan O. Grimalt, Maria Ángeles Martínez, Joaquim Rovira, Marta Schuhmacher.

Enviat a: Environmental Research

Organophosphate metabolite concentrations in maternal urine during pregnancy

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Abstract

The burden of organophosphate (OP) pesticides in pregnant women from Tarragona (n = 54), a Mediterranean area of intense agricultural activity, has been assessed from the study of hydroxylated organic metabolites in urine samples in the three trimesters of pregnancy. 2-Diethylamino-6-methylpyrimidin-4-ol (DEAMPY), a metabolite of pirimiphos, was the compound found in higher concentration, medians 0.66-2.8 μg/g creatinine. 4-Nitrophenol (PNP), a metabolite of parathion, medians 0.24-0.41 μg/g creatinine, was the second most abundant compound. 2-Isopropyl-6-methyl-4-pyrimidol (IMPY), a metabolite of diazinon, was also present but in lower concentrations. Except for DEAMPY, the concentrations found in this cohort were lower than those reported in studies from other countries.

Intraclass correlation coefficients (ICCs) were calculated for the compounds found in more than the 35% of the samples, the reliability between trimesters was poor (<0.40) to fair (0.40-0.60). Statistically significant differences were observed for the creatinine adjusted concentrations of the most abundant OP metabolites in these trimesters when examined with the Wilcoxon signed rank test for paired data.

In general, no association was found between urinary OP metabolites and most demographic and lifestyle predictors. However, a positive significant association was observed for women with vegetarian diet and for women of higher economic status and eventual consumption of organic food which showed higher PNP concentrations. These results broadly indicated that higher fruit and vegetable consumption may involve higher OP pesticide ingestion but the overall association was weak.

Keywords: Organophosphorus pesticides, Human Biomonitoring, Pregnant Women, Maternal exposure.

Introduction

Organophosphate (OP) pesticides are widely used to control insects in agriculture, commercial buildings, gardens and indoor and outdoor domestic environments, involving human exposure. These pesticides have been found in different matrices, such as dietary products, water, outdoor and indoor air and house dust (Mercier et al., 2011, Banerjee et al., 2012; Coscollà et al., 2017; Gibbs et al., 2017; Sousa et al., 2018; Tang et al., 2018). Thus, people can be exposed to OP pesticides as consequence of domestic use, proximity to spraying areas or consumption of contaminated drinks and food (Becker et al., 2006; McKone et al., 2007). Because of their extensive use and potential toxicity, there is concern on the potential negative effects of these compounds in the environment and human health (Barr, 2008). Respiratory, digestive, reproductive and neurological problems, among others, have been related to OP pesticide ingestion or inhalation in humans (Ye et al., 2017; Arcury et al., 2016; Llop et al., 2017; Jokanovic, 2018; Liang et al., 2019).

When OP pesticides enter into the human body, they are metabolized and excreted through the urine, either in free form or bound to glucuronic acid or sulfates (Barr, 2008). Following a two-step metabolic pathway, they are transformed into a dialkylphosphate (DAP) and a hydroxylated organic moiety that is specific of each pesticide (Chambers and Russell, 1995).

Tarragona is a Catalan province of intense agricultural activity and Reus, the city where the participating women were recruited, is located in an area of high olive oil, wine and hazelnut production (Tousa, 2018; Puig-Montserrat et al., 2017). The use of OP pesticides during the last decades might have had an impact on the population living in this area which is particularly important for newborns exposure. The present study is therefore addressed to examine the burden of OP pesticides in pregnant women residing in this area by analysis of urine samples. Furthermore, in view of the metabolic changes undergone by

mothers during pregnancy the study is also addressed to assess the variability of urinary OP pesticides during this period.

Methods

Population and study design

The study population comprises a cohort (EXHES-Spain cohort) of pregnant women (n = 54) that were recruited during their first prenatal visit to the University Hospital "Sant Joan de Reus" (Reus, Catalonia, Spain) as part of the European HEALS project (recruitment started in March 2016 and ended in October 2017). Urine samples were collected in the first, second and third trimester of pregnancy of each women. The inclusion criteria were as follows: older than 16 years, intention to deliver at the reference hospital, and lack of language communication problems. The study was approved by the Ethical Committee of the Clinical Research of the University Hospital "Sant Joan de Reus". Written informed consent was obtained from the participants.

Sample preparation and instrumental analysis

Sample preparation and the procedures of analysis have been described elsewhere (Garí et al, 2018). Briefly, centrifuged and filtered urine samples were introduced into 10-mL centrifuge tubes together with a mixture of isotopically labelled internal standards for hydrolysation with β-glucoronidase. The hydrolysed samples were cleaned up by solid-phase extraction using cartridges preconditioned with a mixture of (1:3) MeOH:acetone followed by a solution of acetic acid 1% in H_2O. The OP metabolites were eluted with a mixture of (1:3) MeOH:acetone. The collected extracts were reduced to near dryness with a gentle N_2 stream and transferred to chromatographic vials with (1:3) MeOH:H_2O.

Identification and quantification of six specific organophosphate metabolites, DEAMPY, IMPY, MDA, PNP, CMHC, TCPY (Table 1), was carried out using an Ultra-Performance Liquid Chromatography (UPLC Acquity H-Class, Waters, Milford, MA, USA) equipped with an electrospray ionization interface. The chromatographic separation was performed on a Betasil C_{18} column.

Table 1. Metabolite's acronyms and principal uses of the pesticides analysed.

Acronym	Analyte	Pesticide	Principal uses	Legal status[1]
DEAMPY	2-diethylamino-6-methylpyrimidin-4-ol	Pirimiphos	All crops, specially fruits and citrus plantations and agricultural facilities	Approved[2]
IMPY	2-isopropyl-6-methyl-4-pyrimidol	Diazinon		Not approved (2007/393)
MDA	Malathion dicarboxylic acid	Malathion		Approved
PNP	4-nitrophenol	Parathion		Not approved (01/520/EC)
TCPY	3,5,6-trichloro-2-pyridinol	Chlorpyriphos		Approved
CMHC	3-chloro-4-methyl-7-hydroxicoumarin	Coumaphos	Farm and domestic animals to control mite	Not approved[3]

[1]European Comission; [2]Pirimiphos-ethyl is not approved (2002/2076); [3]Never notified and authorised in EU.

Synthetic urine was used for blanks, quality control materials and calibration curves. Accuracies were assessed at low and high and concentration levels. Calibration curves were prepared by adding 25 µl of standard solutions at concentrations ranging from 2.5 to 800 ppb into synthetic urine. Quantification was performed by isotopically-labelled internal standards (Garí et al., 2018). Since 2016, this methodology is externally checked by participation in rounds of the German External Quality Assessment Scheme (G-Equas), which includes the organophosphate metabolites PNP and TCPY.

Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). Statistics was focused on the metabolites found above limit of detection in more than 35% of the samples: DEAMPY, IMPY and

PNP. One-half of the limits of detection were assigned to non-detected values. Geometric means and 95% confidence intervals, as well as the medians, were used for the descriptive analysis (Table 4).

The compound concentrations were transformed into natural logarithms for normalization before calculation of intraclass correlation coefficients (ICCs) and multivariate regression models.

To assess variability in urinary concentrations over the three trimesters, ICCs were calculated only for the analytes detected in at least 35% of the samples. For each compound the reliability was characterized as poor (ICC < 0.40), fair (0.40 ≤ ICC < 0.60), good (0.60 ≤ ICC < 0.75) and excellent (≤ 0.75) (Rosner, 2011).

Regression analyses were performed to explore associations between the individual log transformed concentrations of OP pesticides metabolites and several demographic and lifestyle predictors.

Results and discussion

Socio-demographic characteristics

Table 2 summarizes the descriptive characteristics of the women included in the study. The information was obtained from questionnaires administered during recruitment. The average age of the participating women was 33.9 years, ranging between 26 and 45 years. Fifty percent of the women had normal weight, 46% were overweight or obese and only two were underweight.

Most women were predominantly employed (90%), 86% worked indoor and 4% outdoor. Seventy-two percent were transported to work by car. Fifty-seven percent of the participants had a median economic status and 39% had university studies. Nearly all women had most omnivorous diet (96%), 80% of them never or hardly ever bought organic food, and most of them drank bottled water (82%).

Table 2. Demographics of study participants

Characteristics	n (%)
Age (n=54)	33.9±4.9
Pre-pregnancy BMI[a] (n=54)	
Underweight	2 (4)
Normal weight	27 (50)
Overweight	15 (28)
Obese	10 (18)
Economic status (n=54)	
High	13 (24)
Median	31 (57)
Low	10 (19)
Smoking (n=54)	
Never	40 (74)
No (during pregnancy)	10 (19)
Yes (during pregnancy)	4 (7)
Educational level (n=54)	
Primary	15 (28)
Secondary	18 (33)
University	21 (39)
Work (n=51)	
Indoor	44 (86)
Outdoor	2 (4)
Does not work	5 (10)
Transport to work (n=46)	
Car	33 (72)
Walking	11 (24)
Bus	2 (4)
Diet (n=54)	
Omnivorous	52 (96)
Vegetarian	2 (4)
Water (n=49)	
Bottled	40 (82)
Tap	5 (10)
Both	4 (8)
Organic food (n=51)	
Never	29 (57)
Hardly ever	12 (23)
Sometimes	8 (16)
Very often	2 (4)

[a]Body mass index

Concentrations of urinary organophosphate metabolites

Descriptive statistics of the urinary concentrations of the analysed metabolites are summarized in Table 3. The detection frequencies of each compound ranged from below limit of detection (<LOD) to 79% in the first trimester, <LOD-80% in the second and <LOD-98% in the third. The most frequently detected compound in all cases was DEAMPY, the pirimiphos metabolite, followed by PNP, the parathion metabolite. TCPY, the chlorpyrifos metabolite, was found at extremely low detection frequency.

In all three trimesters the metabolite found in highest concentration was DEAMPY, medians 2.6, 1.4 and 4.0 ng/g creatinine, for the first, second and third trimester, respectively. The second most abundant was PNP, again in the three trimesters, medians 0.44, 0.37 and 0.26 ng/g creatinine, respectively. The

occurrence of this parathion metabolite is unexpected as the use of this pesticide in Europe is not approved (European Commission, 2001).

Comparison of the results of the present cohort with those from other adult population studies show low values of all metabolites except for DEAMPY (Table 4). The high concentrations of DEAMPY in Tarragona are comparable with those from the rural population of Catalonia and Atlanta. The median of this metabolite was lower in Galicia and Valencia (0.43 and 0.03 ng/mL, respectively). In this study it has not been possible to define the source of exposure of DEAMPY metabolite. However, Catalonia has high levels of cereals production and pirimiphos, the parent compound of DEAMPY, is used for the protection of stored grain.

The median concentration of PNP, 0.3 ng/mL, the second most abundant metabolite in our study, is similar to those found in Puerto Rico and US (NHANES), 0.5 ng/mL. This compound was found in much higher concentrations in urines of Catalonia, Galicia, Greece, Vietnam, China and some US sites, 1.6-5.0 ng/mL (Table 4). TCPY was not found in the cohort of Tarragona despite chlorpyrifos, the precursor pesticide, is largely applied in many world areas. Other studies performed in Spain, Valencia, Galicia and Catalonia showed higher concentrations of this metabolite (0.49, 2.4 and 0.93 ng/mL, respectively) than in Tarragona.

Intraclass correlation coefficient

ICCs were calculated for the metabolites detected in at least 35% of the samples, DEAMPY, IMPY and PNP (Table 5). Both uncorrected and creatinine adjusted data were used, showing ICC ranges of 0.28-0.48 and 0.26-0.43, respectively. The differences between these two series of data were only significant for PNP ($p<0.05$). According to the observed coefficients, the reproducibility of urinary OP metabolite concentration across these three trimesters ranged between poor for DEAMPY and non-adjusted PNP and fair for adjusted PNP and IMPY.

Table 3. Creatinine adjusted (μg/ g creatinine) and non-adjusted urinary levels (ng/mL) of OPs metabolites in the first, second and third trimester of pregnancy. Full names of the metabolites in Table 1.

			Creatinine adjusted results				Non-adjusted results				p-value[4]
Metabolite	**LOD[1] (ng/mL)**	**DF[2] (%)**	**GM**	**(CI)**	**Median**	**Range**	**GM**	**(CI)**	**Median**	**Range**	
1st Trimester n=53											
DEAMPY	0.017	79	1.6	(0.76-3.3)	2.6	<LOD-84	0.82	(0.39-1.7)	1.8	<LOD-67	**0.023**[a]
IMPY	0.014	38	0.049	(0.027-0.088)	<LOD	<LOD-58	0.025	(0.015-0.044)	<LOD	<LOD-11	0.67[a]
MDA	0.069	6	<LOD		<LOD	<LOD-8.2	<LOD		<LOD	<LOD-12	
PNP	0.017	79	0.44	(0.25-0.78)	0.75	<LOD-9.0	0.23	(0.13-0.40)	0.41	<LOD-6.4	1.0[a]
TCPY	0.02	4	<LOD		<LOD	<LOD-0.95	<LOD		<LOD	<LOD-0.15	
CMHC	0.026	0	<LOD		<LOD	-	<LOD		<LOD	-	
2nd Trimester n=54											
DEAMPY	0.017	80	0.89	(0.48-1.7)	1.4	<LOD-52	0.42	(0.21-0.83)	0.66	<LOD-133	**0.0094**[b]
IMPY	0.014	41	0.066	(0.035-0.13)	<LOD	<LOD-36	0.031	(0.017-0.057)	<LOD	<LOD-31	0.98[b]
MDA	0.069	2	<LOD		<LOD	<LOD-0.99	<LOD		<LOD	<LOD-0.39	
PNP	0.017	74	0.37	(0.20-0.69)	0.56	<LOD-9.1	0.17	(0.091-0.33)	0.24	<LOD-12	**0.050**[b]
TCPY	0.02	0	<LOD		<LOD	-	<LOD		<LOD	-	
CMHC	0.026	0	<LOD		<LOD	-	<LOD		<LOD	-	
3rd Trimester n=50											
DEAMPY	0.017	98	4.3	(2.7-6.8)	4.0	<LOD-232	3.1	(1.8-5.2)	2.8	<LOD-179	0.82[c]
IMPY	0.014	46	0.067	(0.035-0.13)	<LOD	<LOD-5.9	0.048	(0.025-0.093)	<LOD	<LOD-4.3	0.31[c]
MDA	0.069	0	<LOD		<LOD	-	<LOD		<LOD	-	
PNP	0.017	78	0.26	(0.15-0.46)	0.47	<LOD-8.4	0.19	(0.10-0.34)	0.31	<LOD-6.4	**0.0032**[c]
TCPY	0.02	0	<LOD		<LOD	-	<LOD		<LOD	-	
CMHC	0.026	0	<LOD		<LOD	-	<LOD		<LOD	-	

[1]LOD: Limit of detection; [2]DF: Detection frequencies; [3]GM (CI): Geometric mean and 96% confidential interval; [4]p-values of the Wilcoxon signed rank test for paired data evaluating differences between trimesters, creatinine adjusted results were used (in bold, statistically significant differences). [a]First trimester vs. second trimester; [b]Second trimester vs. third trimester; [c]First trimester vs. third trimester.

Table 4. Comparison of the 50th percentile data (ng/mL) in mother's urine samples from Tarragona with those of other similar studies. The results included in this Table are the arithmetic means of the medians of the three trimesters.

	N	Population	Year	DEAMPY	IMPY	MDA	PNP	TCPY	Study
Tarragona (Spain)	54	Women[2]	2016-2017	1.8 [1.4]	<LOD[1] [0.06]	<LOD	0.3 [0.2]	<LOD	*Present study*
Valencia (Spain)	573	Women[2]	2003-2006	[0.03]	[0.03]		[0.04]	[0.49]	*Llop et al., 2017*
Catalonia (Spain)	42	General	2016	1.1	<LOD	<LOD	1.8	2.4	*Garí et al., 2018*
Galicia (Spain)	21	General	2016	0.43	<LOD	<LOD	1.0	0.93	*Garí et al., 2018*
Greece	40	General	2010-2014		0.3 [0.3]	0.4 [0.4]	1.6 [1.6]	6.1 [5.5]	*Li and Kannan, 2018*
Queensland (Australia)	200	Women	2012-2013		[0.34]	[0.81]	[1.3]	[17]	*Heffernan et al., 2016*
US (NHANES)	355	Women	2009-2010		<LOD	<LOD	0.5		*Lewis et al., 2015*
Puerto Rico (US)	152	Women[2]	2010-2012		<LOD	<LOD	0.5		*Lewis et al., 2015*
Altanta (US)	55	General	2012	1.0	0.75	1.2	1.4	1.9	*Davis et al., 2013*
Rotterdam (Netherlands)	100	Women[2]	2002-2006					1.2	*Ye et al., 2008*
China	86	General	2010-2014		0.2 [0.2]	0.6 [0.5]	5.0 [5.0]	3.4 [3.7]	*Li and Kannan, 2018*
Vietnam	22	General	2010-2014		0.2 [0.2]	0.4 [0.5]	2.6 [2.6]	9.3 [9.3]	*Li and Kannan, 2018*

[1]<LOD data below limit of detection; [2]Pregnant women. Geometric mean into [ ].

These observed ICCs are consistent with those reported in a study on urinary PNP from Puerto Rican women during pregnancy (Lewis et al., 2015) in which ICCs of 0.31 and 0.28 were observed for uncorrected and SG (specific gravity) adjusted levels, respectively. Other studies have assessed different time ranges. Thus, Cequier et al. (2017) and Egeghy et al. (2011) found moderate ICC values for all compounds, in a comparison of the diurnal variation. Studies encompassing longer time spans, e.g., 18 weeks (Spaan et al., 2015) or one year (Attfield et al., 2014), found OP metabolite ICCs ranging from 0.08 to 0.38. According to these ICC values, comparison of urinary metabolites between different time periods requires the analysis of more samples as longer is the time span considered.

Table 5. Creatinine adjusted and non-adjusted interclass correlation coefficients (ICC, 95% CI) of urinary concentrations of OP pesticide metabolites in pregnant women from Tarragona.

OP metabolite	ICC (95% CI)	
	Creatinine adjusted	**Non-adjusted**
All participants (n=48)		
DEAMPY	0.26 (-0.13-0.54)	0.32 (-0.024-0.58)
PNP	0.43 (0.088-0.66)	0.28 (-0.17-0.57)
IMPY	0.43 (0.082-0.66)	0.48 (0.16-0.69)

Further insight into the homogeneity of the data was evaluated from the Wilcoxon signed rank test for paired data on the creatinine adjusted concentrations. Significant differences were observed for the OP metabolites found in higher levels (Table 3). That is, in DEAMPY the values of the second trimester and in PNP the values of the third trimester were statistically significantly lower than the concentrations of the same metabolites in the other pregnancy periods (Table 3). These differences found between trimesters could be caused by metabolism variations or life-style changes during pregnancy. Pregnant women tend to raise their consumption of vegetables and fruits, increasing their potential exposure to OP pestidices. The values of the third

trimester were higher for DEAMPY, the OP metabolite found in highest concentrations.

Influence of demographic and lifestyle predictors on urinary OP metabolites

Demographic and lifestyle data were obtained from a questionnaire administered to each participant. The number of statistically significant associations was low. No relationship was found between urinary OP metabolites and several demographic and lifestyle predictors, such as educational level, smoking during pregnancy, working indoor or outdoor, transport to work, type of water consumption or BMI. No significant association was found for DEAMPY concentrations, the major OP metabolite, and any of the socio-demographic and life-style predictors.

Regression analysis showed a positive significant association ($p<0.05$) between women with vegetarian diet and higher IMPY concentrations (diazinon metabolite; Table 6). This association has also been observed in other studies (Aprea et al., 1999 and Berman et al., 2016) and is consistent with the use of OP pesticides for plant protection against insects in agriculture.

A positive significant association ($p>0.01$) between PNP concentrations and eventual consumers of organic food was also observed. Other studies, encompassing children or adults, found associations between organic food consumption and OP pesticide exposure markers, either specific organic moieties or DAPs (Curl et al., 2003; Lu et al., 2006; Lu et al., 2008; Oates et al., 2014 and Berman et al., 2016). The Tarragona results may be explained by higher consumption of organic food among vegetarians or vegans (Baudry, 2016). However, the association is not very significant because it is not observed in the mothers who consumed organic food very often (Table 6). In any case, the positive statistically significant association between concentrations for this pesticide and women in higher economic status ($p<0.05$;

Table 6) is consistent with the previous associations as people of higher income tend to have greater awareness of the need of good nutrition and consume more fruits and vegetables (Kamphuis et al., 2006). Associations between higher urinary concentrations of OP metabolites, DAPs in this case, in the higher income class have also been observed in Canada (Socolof et al., 2016).

Table 6. Results of simple linear regression models with demographic and lifestyle predictors and urinary concentrations of organophosphate metabolites.

Variable	Categories[1]	*DEAMPY*		*PNP*		*IMPY*	
		β[2]	*p-value*	β[2]	*p-value*	β[2]	*p-value*
Pre-pregnancy BMI	Underweight	Ref.					
	Normal weight	1.31	0.18	-0.11	0.91	-0.79	0.39
	Overweight	1.91	0.056	-0.88	0.34	-0.94	0.32
	Obese	1.37	0.18	0.090	0.93	-0.79	0.42
Diet	Omnivorous	Ref.					
	Vegetarian	-0.49	0.61	-0.62	0.49	2.4	**0.012**
Organic food	Never	Ref.					
	Hardly ever	0.083	0.86	0.41	0.34	-0.42	0.37
	Sometimes	1.01	0.072	1.3	**0.0098**	-0.14	0.80
	Very often	0.67	0.50	0.25	0.78	-0.47	0.63
Drink water	Bottled	Ref.					
	Tap	-0.34	0.60	0.52	0.36	-0.94	0.15
	Both	0.76	0.29	1.2	0.060	-0.096	0.89
Economic status	High	Ref.					
	Median	0.59	0.19	0.84	**0.045**	-0.48	0.26
	Low	0.96	0.10	0.68	0.20	-0.15	0.78
Educational level	Primary	Ref.					
	Secondary	0.38	0.42	-0.71	0.1	-0.31	0.49
	University	0.045	0.92	-0.19	0.65	-0.46	0.29
Smoking	Never	Ref.					
	No (pregnancy)	-0.052	0.92	-0.21	0.64	0.41	0.37
	Yes (pregnancy)	-0.10	0.89	-0.83	0.20	0.87	0.19
Work	Indoor	Ref.					
	Outdoor	0.30	0.77	1.6	0.1	0.81	0.42
Transport to work	Car	Ref.					
	Walking	0.25	0.59	-0.37	0.38	-0.17	0.70
	Bus	0.18	0.85	-0.08	0.93	-1.4	0.14

[1]Same categories as Table 1; [2]β Coefficients of the simple regression models with non-standarized variables. In bold, statisticaly significant relations

Conclusions

Comparison of the concentrations of urinary OP metabolites in the pregnant women from the Tarragona area with those reported in previous studies show low values except for DEAMPY, the pirimifos metabolite, the most abundant of this cohort. TCPY, the metabolite of chlorpyrifos, was not observed in nearly all cases despite this OP pesticide is still allowed for use. Moderate variability in the three trimesters of pregnancy was assessed by ICC calculation of almost all OP pesticides. Statistically significant differences were observed for the creatinine adjusted concentrations of the most abundant OP metabolites in these trimesters when examined with the Wilcoxon signed rank test for paired data. Accordingly, more than one spot of urine sample may be needed to characterize exposures over pregnancy.

In general, no association was found between urinary OP metabolites and most demographic and lifestyle predictors. However, a positive significant association was observed for women with vegetarian diet and for women of higher economic status and eventual consumption of organic food, which showed higher PNP concentrations. These results broadly indicate that higher fruit and vegetable consumption may involve higher OP pesticide ingestion but the overall association is weak.

Acknowledgements

This paper was supported by funding from the European Union projects: HEALS (FP7-ENV-2013- 603946), Neurosome (H2020-MSCA-ITN-2017 SEP-210411486) and EDC-MET (H2020-HEALTH/0490-825762). J. Rovira received funds from Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016-2020).

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4.4 Discussió dels resultats

En aquest capítol s'han presentat alguns dels pesticides més utilitzats en els últims anys, alguns d'ells prohibits i altres en ús. S'ha comprovat mitjançant l'anàlisi d'orina que tant nens com adults hi estan exposats, ja estiguin laboralment exposats o no.

Els resultats principals que s'han assolit en aquesta tesi s'han mostrat en forma de quatre publicacions científiques, dos d'elles publicades i les altres dos enviades. En aquesta secció es dona una visió global del conjunt de resultats obtinguts. A més, la discussió d'aquest capítol també es complementa amb la inclusió d'altres resultats complementaris encara no publicats per proporcionar una informació completa dels resultats obtinguts.

Desenvolupament i aplicació d'una metodologia analítica nova per l'anàlisi de pesticides OPs i PYR

En el model de vida actual l'ús de productes fitosanitaris (pesticides o herbicides entre d'altres) és imprescindible per a la producció agrícola, tant en els sistemes convencionals d'agricultura com en d'altres. Sovint els mètodes d'anàlisi existents queden obsolets perquè els productes que s'utilitzaven fa uns anys no són els mateixos que s'utilitzen ara. En el context d'aquesta tesi doctoral s'ha desenvolupat una metodologia analítica que ens permet determinar alguns dels metabòlits més importants dels pesticides OPs i PYR.

El mètode desenvolupat es descriu a l'ARTICLE 4. Aquesta metodologia ha permès identificar i quantificar inequívocament els analits escollits en diferents tipus de poblacions. Així doncs, s'ha aplicat amb èxit en l'anàlisi de les mostres d'orina presentades als ARTICLES 4, 5 i 6, que pertanyien a nens, dones i població general exposada i no exposada.

Per contra, la metodologia analítica desenvolupada no va permetre determinar la concentració de piretroides de les dones embarassades d'altres zones que tampoc permetia l'anàlisi dels PYRs. S'ha conclòs que degut als canvis hormonals de l'embaràs, l'orina de les dones embarassades conté alguún composto que interfereix amb els ions dels piretroides.

Concentracions de pesticides OP i PYR en les poblacions d'estudi

A la Figura 4.18 es comparen les cinc regions estudiades. Encara que el més acurat és utilitzar els resultats ajustats, com s'ha explicat a la secció de mètodes, en part de la discussió s'ha decidit utilitzar els valors sense ajustar (ng/mL) per no tenir disponibilitat de la creatinina o la densitat per ajustar totes les mostres de la mateixa manera.

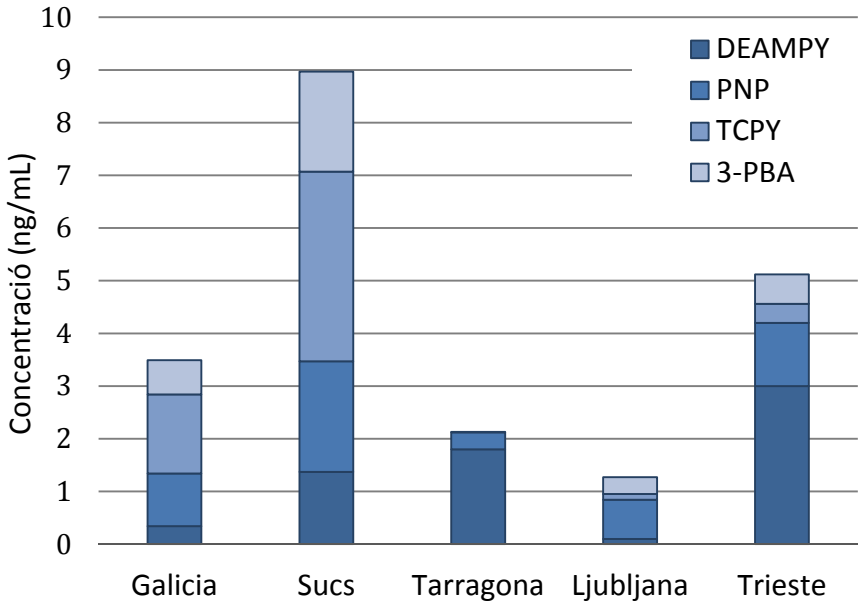

Figura 4.18 Mediana de la concentració de pesticides OPs i PYR en les diferents regions estudiades.

En conjunt la població més exposada és la de Sucs (Catalunya), aquest és un resultat esperable ja que part dels voluntaris són treballadors agrícoles que utilitzen pesticides en el seu dia a dia. A continuació els que tenen

concentracions més elevades són els nens de Trieste seguits de la població general de Galícia (conjunt de població rural i urbana) i per últim els que tenen concentracions més baixes són les dones de Tarragona i les parelles de mares i nens de Ljubljana. Aquest darrer resultat està en consonància amb el fet que Eslovènia és un dels països que menys pesticides consumeix a nivell Europeu (Eurostat, 2018).

Als llocs presumptament exposats, Galícia i Sucs, el compost que s'ha trobat en major concentració ha estat el TCPY, metabòlit del pesticida clorpirifos, un dels més utilitzats en l'actualitat. Mentre en les regions on se suposa una exposició majoritària per dieta els més abundants han estat el PNP i el DEAMPY, metabòlits de pirimifos i paratió, respectivament.

L'exposició en cada població pot diferir significativament entre elles depenen de l'estil de vida o els factors socioeconòmics. A la Figura 4.19 es comparen totes les mostres de totes les poblacions analitzades en aquesta tesi, als ARTICLES 5, 6 i 7 es comparen aquests mateixos resultats amb estudis d'arreu del món.

Si només tenim en compte la població general (Carbia, Santiago de Compostel·la, Sucs, Tarragona i les dones de Ljubljuana), les concentracions del metabòlit TCPY a les poblacions de Carbia i Sucs, ambdues regions rurals, són notablement superiors que les d'aquells que viuen a Santiago de Compostel·la, Ljubljana o Tarragona. Aquesta tendència també s'observa amb el metabòlit de piretroides 3-PBA i amb el PNP.

Per contra el DEAMPY (metabòlit del pirimifos) té unes concentracions molt elevades en les dones embarassades de Tarragona, superant les dels treballadors agrícoles de Sucs, mentre que els nivells de la resta de compostos són molt baixos. En estudis anteriors s'ha vist que, normalment, d'entre tota la població general les concentracions de pesticides OPs i PYR en dones embarassades tendeixen a ser més baixes que les de població general o

exposada (Katsikantami et al., 2019). Aquest resultat anormal de DEAMPY pot ser degut a una exposició puntual a Tarragona o a uns hàbits alimentaris diferents. El pirimifos és un pesticida utilitzat per l'emmagatzematge de cereals, un grup d'aliments molt consumit en la dieta mediterrània.

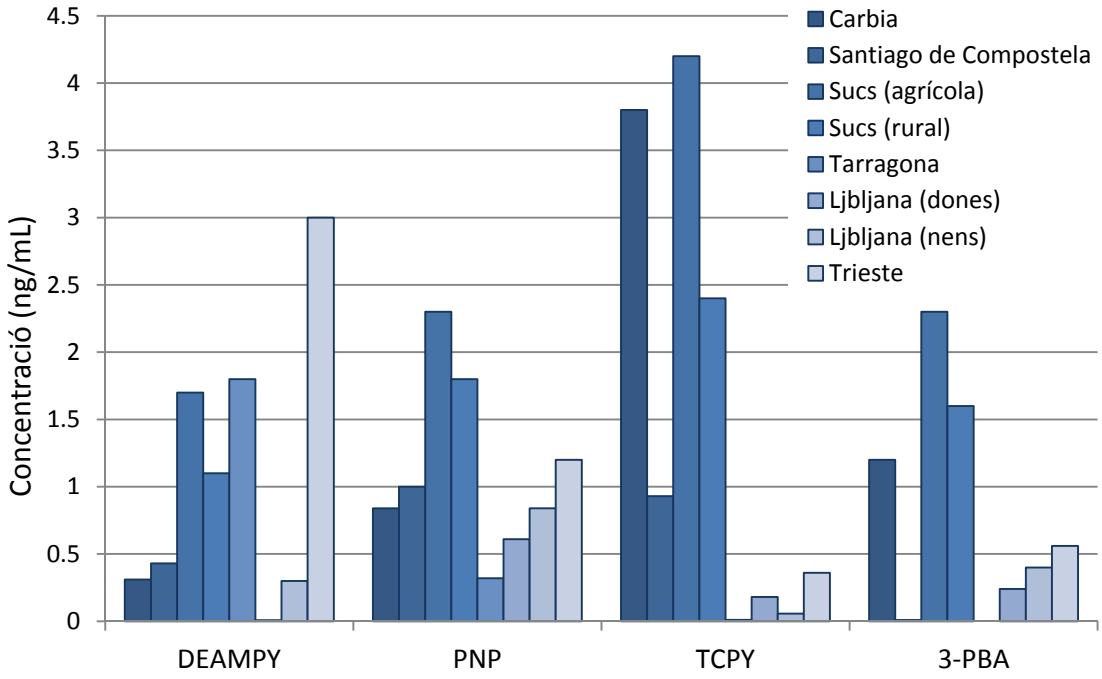

Figura 4.19 Comparació de la mediana de la concentració de pesticides OPs i PYR entre totes les poblacions estudiades en la tesi.

En principi els nens es podrien considerar del grup de població general ja que la seva exposició a pesticides OPs i PYR és produeix, com en els adults, mediambientalment o per la dieta. No obstant això, són un grup més sensible degut a tenir un pes corporal baix i presenten riscos de salut més alts perquè el seu organisme està en desenvolupament. De la mateixa manera que en els adults, els nens de Trieste tenen concentracions més altes que els de Ljubljana, a més en el cas del PNP s'han trobat concentracions superiors que els tots els estudis amb adults exceptuant els de Sucs.

Tal com es comentava en el cas de les dones embarassades de Tarragona, els nivells de DEAMPY són anormalment alts en els nens de Trieste. Com es

discuteix a l'ARTICLE 5, encara que en aquest estudi no s'ha pogut determinar la font d'exposició a pirimifos un estudi en el que analitzaven la distribució de pesticides en la província de Bolonya (Nord d'Itàlia) va trobar el pirimifos entre un dels compostos més abundants (Ghini et al., 2004).

Un punt a destacar en aquesta comparació, discutida en més detall a l'ARTICLE 6, és la diferència entre les dones i els nens d'Eslòvenia. En tots els metabòlits excepte el TCPY els nens presenten concentracions a l'orina significativament més elevades. En aquest cas en particular es pot considerar que l'exposició de cada mare amb el seu fill és similar, per tant, l'explicació d'aquestes diferències no es deu a una font d'exposició diferent. Encara que l'explicació a aquest fet no s'ha investigat directament un dels motius d'aquesta diferència podria ser que els nens tinguessin un metabolisme més efectiu de cara a l'eliminació de pesticides OPs i PYR o que la quantitat de pesticida ingerit pels nens en relació al seu pes és major que en les seves mares.

Influència de factors sociodemogràfics en l'exposició a pesticides OP i PYR

En la secció anterior s'ha esmentat que existeixen diversos factors que propicien l'exposició a pesticides organofosforats i piretroides. Aquests s'estudien en els ARTICLES 5, 6 i 7. A la Taula 4.1 es presenten alguns dels factors que poden influir, tot i que aquests no són tan determinants com en el cas dels compostos persistents (Capítol 3).

A l'estudi amb nens d'Itàlia (ARTICLE 5) es va veure que l'educació familiar estava relacionada amb la concentració en orina del metabòlit DEAMPY, mentre que aquesta associació no es veia ni a l'estudi d'Eslovènia ni de Tarragona. Una educació materna baixa també es va relacionar amb nivells baixos del metabòlit TCPY a Nova York (Berkowitz et al., 2003). Diferències similars també es van publicar en estudis en dones de Cincinnati i del Canada, els de nivell educatiu

major tenien nivells més alts de pesticides OP (Yolton et al., 2013; Sokoloff et al., 2016). Sovint aquests diferencies s'associen a diferents hàbits alimentaris o culturals, tot relacionant-los amb un consum més alt de productes tractats amb pesticides OPs.

Tenir un pes normal té una relació significativament negativa amb la concentració del metabòlit TCPY en les mares de Ljubljana, però no s'ha vist aquest efecte en la resta d'estudis. En un estudi amb dones dut a terme al Canada (Sokoloff et al., 2016) també es va observar aquesta mateixa relació.

Com ja s'ha dit, un dels determinants més importants de les concentracions d'aquests pesticides és viure en un entorn urbà, rural o treballar en el camp. Les darreres els tenen en més concentració. Aquests resultats coincideixen amb els observats en molts altres estudis arreu del món (Aprea et al., 2005; Curwin et al., 2007; Phung et al., 2012; Koureas et al., 2014).

Només en el cas de Sucs i les diferències entre mares i nens de Ljubljana s'ha vist una relació amb l'edat. En el cas de Sucs els voluntaris presenten concentracions més altes de tots els metabòlits excepte el DEAMPY amb l'edat. En canvi, en la cohort de Ljubljana els nens tendeixen a tenir concentracions més altes que les seves mares. Mentre que les diferències entre mares i nens es poden deure a un diferent metabolisme entre nens i adults (ARTICLE 6), les diferències per edat que s'observen en la població general de Sucs, on part són treballadors agrícoles, suggereixen un altre patró d'exposició. Per exemple, una mala manipulació dels pesticides, un ús incorrecte dels equips de protecció individual (EPI) o falta d'higiene a l'hora d'acabar de fumigar que poden influir en l'exposició. A continuació s'avaluen alguns d'aquests aspectes en la població agrícola de Sucs.

Taula 4.1 Diferències en la mediana de la concentració ajustada per creatinina (μg/g creatinina) dels metabòlits principals per les característiques de les diferents poblacions.

Característiques	DEAMPY		PNP		TCPY		3-PBA	
	P50	IQR[1]	P50	IQR	P50	IQR	P50	IQR
Ljubljana (nens; n=164)								
Sexe								
Nens (n=83)	0,83	0,13-2,2	1,4	0,65-2,8	0,054	0,028-0,47	0,80	0,31-1,6
Nenes (n=80)	0,29	0,055-1,3	1,4	0,68-2,92	0,13	0,039-0,83	0,89	0,14-2,0
IMC[2]								
Normal (n=127)	0,38	0,080-1,4	1,4	0,66-2,9	0,099	0,033-0,66	0,82	0,12-1,6
Sobrepès/Obesitat (n=32)	0,70	0,15-2,6	1,3	0,67-2,5	0,076	0,028-0,31	0,87	0,56-1,8
Educació familiar								
Sota universitat (n=66)	0,40	0,11-1,5	1,5	0,80-3,0	0,076	0,038-0,45	0,78	0,15-1,6
Universitat o més (n=97)	0,46	0,070-2,2	1,3	0,64-2,9	0,12	0,025-0,66	0,87	0,25-1,9
Ljubljana (mares; n=167)								
Edat								
< 40 anys (n=106)	0,055	0,022-1,0	1,2	0,62-2,9	0,50	0,15-1,8	0,65	0,036-1,2
≥ 40 anys (m=61)	0,036	0,013-1,6	1,1	0,54-2,3	0,31	0,069-1,3	0,59	0,023-1,2
IMC[2]								
Normal (n=112)	0,049	0,019-1,02	1,1	0,57-3,2	**0,57***	**0,16-1,8**	0,65	0,044-1,3
Sobrepès/Obesitat (n=56)	0,058	0,015-1,4	1,1	0,63-2,1	**0,29***	**0,055-0,92**	0,58	0,025-1,1
Educació mare								
Sota universitat (n=75)	0,042	0,019-1,2	1,1	0,57-2,5	0,40	0,057-1,7	0,64	0,16-1,2
Universitat o més (n=93)	0,066	0,016-1,1	1,2	0,61-3,1	0,51	0,12-1,3	0,59	0,026-1,3
Consumeix tabac								
Si (n=27)	0,099	0,035-1,7	0,92	0,41-2,9	0,56	0,24-1,2	1,1	0,025-1,6
No (n=141)	0,044	0,016-1,0	1,1	0,62-2,6	0,43	0,082-1,6	0,61	0,035-1,1
Trieste (n=198)[3]								
Educació familiar								
Sota universitat (n=106)	**2,8***	**0,96-8,5**	1,3	0,52-3,2	0,43	0,059-1,4	0,49	0,069-1,0
Universitat o més (n=91)	**6,1***	**1,5-16**	1,8	0,75-3,7	0,39	0,083-1,3	0,70	0,16-1,8
Galícia (n=37)								
Edat								
≤ 50 anys (n=14)	0,51	0,013-2,3	0,90	0,52-1,1	1,6	0,69-4,1	0,65	0,0085-1,5
> 50 anys (m=23)	0,23	0,0096-1,2	0,96	0,58-2,2	1,7	0,67-2,8	0,92	0,0076-1,4
Sexe								
Homes (n=15)	0,62	0,0097-1,7	0,90	0,56-2,3	2,2	0,83-4,5	1,0	0,28-1,9
Dones (n=22)	0,28	0,012-1,1	0,96	0,54-1,2	1,4	0,68-2,3	0,63	0,068-1,1
Residència								
Carbia (n=21)	0,28	0,011-1,4	1,1	0,45-2,1	**2,8***	**2,0-5,9**	**1,3***	**0,92-2,1**
Santiago de Compostel·la (n=16)	0,33	0,010-1,5	0,76	0,59-1,1	**1,2***	**0,32-1,7**	**0,011***	**0,0061-0,83**
Sucs (n=85)								
Edat								
≤ 50 anys (n=46)	1,0	0,49-2,2	**1,3***	**0,88-2,2**	**2,3***	**1,4-3,7**	**1,3***	**0,81-2,2**
> 50 anys (m=39)	1,1	0,15-3,4	**2,0***	**1,1-3,4**	**3,2***	**2,0-5,4**	**2,1***	**1,3-2,9**
Sexe								
Homes (n=58)	1,2	0,15-2,1	1,6	1,1-2,7	3,0	1,5-3,5	1,7	0,98-2,89
Dones (n=27)	0,71	0,55-2,5	1,7	0,95-2,4	2,9	1,6-4,9	1,5	0,88-2,2
IMC[1]								
Normal (n=31)	1,2	0,71-2,7	1,3	0,88-2,4	2,9	1,5-3,7	2,0	0,84-2,7
Sobrepès/Obesitat (n=52)	0,96	0,099-2,51	1,7	1,1-2,8	2,9	1,6-4,4	1,6	1,1-2,8
Treballador agrícola								
Si (n=44)	1,15	0,45-2,5	1,7	1,2-3,0	**3,3***	**1,7-5,1**	1,7	1,0-3,0
No (n=41)	0,87	0,24-2,4	1,5	0,88-2,2	**2,4***	**1,4-3,6**	1,4	0,9-2,3

[1]IQR: Amplitud interquartílica (P25-P75);[2]IMC: Índex de massa corporal; [3]Resultats ajustats per la densitat; * *p-valor* <0,05 pel test Mann-Whitney

Avaluació de l'exposició a pesticides OP i PYR en una població agrícola

Característiques de la població

La població agrícola exposada de Sucs està representada per 44 homes amb edats compreses entre els 22 i 81 i una mitjana de 51,5 anys. A la Taula 4.2 es mostren algunes de les característiques més importants, així com algunes dades sobre la manipulació de pesticides i piretroides.

Taula 4.2 Dades demogràfiques, de manipulació i seguretat de fitosanitaris dels participants de l'estudi

	Participants n (%)		**Participants n (%)**
Edat (n=44)	51,5±15,5	**EPI[2] barreja (n=44)**	
IMC[1] (n=44)		Guants	
Normal	15 (34)	Sempre	20 (45)
Sobrepès	19 (43)	A vegades	10 (23)
Obesitat	10 (23)	Mai	14 (32)
Tipus de cultiu (n=35)		Semimàscara (boca)	
Fruiter	23 (66)	Sempre	12 (27)
Horta	9 (26)	A vegades	12 (27)
Fruiter/horta	3 (8)	Mai	20 (45)
Eines o transport (n=44)		Gorra o barret	
Tractor	35 (80)	Sempre	18 (41)
Motxilla manual	7 (16)	A vegades	4 (9)
Polvoritzador hidraúlic	2 (4)	Mai	22 (50)
Tipus de tractor (n=35)		**EPI[2] aplicació (n=44)**	
Amb cabina	28 (80)	Semimàscara (boca)	
Sense cabina	7 (20)	Sempre	5 (11)
Àrea (n=44)		A vegades	4 (9)
≤ 20 ha	22 (50)	Mai	35 (80)
> 20 ha	22 (50)	Gorra o barret	
Fumigació (n=44)		Sempre	12 (27)
≤ 20 dies/any	25 (57)	A vegades	2 (5)
> 20 dies/any	19 (43)	Mai	30 (68)

[1]Índex de massa corporal; [2]Equip de protecció individual

Més de la meitat dels treballadors agrícoles es dediquen al cultiu d'arbres fruiters (66%), la majoria (80%) d'ells utilitzen un tractor per fumigar els camps, 28 dels quals utilitzen un tractor amb cabina, la resta utilitza un polvoritzador hidràulic o una motxilla. La meitat dels treballadors fumiga camps de més de 20 hectàrees i un 43% ho fa durant més de 20 dies a l'any.

Sobre els equips de protecció individual (EPI) se'ls va preguntar si els utilitzaven durant la barreja i l'aplicació. Durant la barreja menys de la meitat (45%) utilitzen guants a l'hora de manipular els fitosanitaris, l'altre meitat els utilitzen a vegades (23%) o mai (32%). En quant a protecció de les vies respiratòries, només el 27% dels treballadors utilitzen semimàscara sempre, mentre que un 27% l'utilitza a vegades i fins a un 45% no l'utilitza mai. Un 50% utilitza sempre o a vegades gorra o barret. Durant l'aplicació la majoria no utilitza ni semimàscara (80%) ni gorra o barret (68%) mai.

A més de la informació presentada a la Taula 4.2, al qüestionari es preguntava sobre hàbits durant la manipulació com rentar-se les mans, dutxar-se després de l'aplicació o fumar durant l'aplicació. També sobre la utilització d'altres equips de protecció individual, per exemple, roba impermeable, màscares integrals, ulleres, botes de goma o casc respiratori. Els resultats, no es mostren a la taula perquè la majoria tenia els mateixos hàbits durant la manipulació i del 95 al 100% dels treballadors participants a l'estudi no utilitzaven la resta d'EPIs.

Influència de la manipulació de fitosanitaris i la utilització d'equips de protecció individual

A la Taula 4.3 es mostren els resultats de les diferències en la mediana de la concentració ajustada per creatinina (μg/g creatinina) dels metabòlits principals per alguns dels deteminants que poden influir en l'exposició a pesticides OPs i PYR.

De tots els possibles factors avaluats només és significativa la diferència entre utilitzar un tractor amb cabina o sense pel metabòlit DEAMPY, els que no utilitzen cabina tenen més concentració. En aquest cas concret, dels 7 treballadors que utilitzen tractor sense cabina, 3 d'ells mai utilitzen una semimàscara durant l'aplicació, 2 ho fan sempre i els altres 2 a vegades.

Fer servir un EPI adient per a cada activitat és imprescindible quan es treballa amb productes químics com els pesticides OPs i els PYR. Aquests compostos, a part de la dieta, també s'incorporen al cos a través de la respiració i la pell, per tant és molt important protegir-los durant la manipulació.

Taula 4.3 Diferències en la mediana de la concentració ajustada per creatinina (µg/g creatinina) dels metabòlits principals per determinants d'exposició de pesticides OP i PYR a la població agrícola de Sucs.

Determinants	DEAMPY		PNP		TCPY		3-PBA	
	P50	IQR[1]	P50	IQR	P50	IQR	P50	IQR
Tipus cultiu								
Fruiter (n=23)	1,1	0,55-1,9	1,5	1,1-2,1	2,6	1,8-4,1	1,4	1,1-3,8
Horta (n=9)	1,3	0,62-1,6	1,6	1,2-4,6	4,0	3,2-5,0	1,7	0,93-2,9
Eines o transport								
Tractor (n=35)	1,3	0,6-2,6	1,7	1,2-2,6	3,1	1,6-4,7	1,4	1,0-3,4
Motxilla (n=7)	0,8	0,03-1,2	1,2	1,8-2,9	4,0	3,6-5,5	1,7	1,2-2,6
Polvoritzador hidràulic (n=2)	3,7	1,8-5,5	2,3	1,1-3,4	4,2	2,8-5,5	2,2	2,1-2,3
Tipus de tractor								
Amb cabina (n=28)	**1,0***	**0,47-2,2**	1,6	1,1-2,1	3,3	1,7-4,8	1,4	1,0-3,2
Sense cabina (n=7)	**2,5***	**1,4-7,0**	5,4	1,6-6,6	2,9	2,1-4,1	1,9	1,5-4,4
Àrea								
≤ 20 ha (n=50)	1,2	0,28-2,0	1,6	1,2-3,5	3,5	1,8-5,4	2,3	1,3-3,8
> 20 ha (n=50)	1,1	0,42-2,9	1,8	1,1-2,2	3,1	1,8-4,2	1,4	0,98-2,7
Fumigació								
≤ 20 dies/any (n=57)	1,3	0,77-3,0	1,7	1,2-2,8	3,8	1,7-6,9	2,3	1,0-3,0
> 20 dies/any (n=43)	0,8	0,013-2,0	1,5	1,1-2,9	3,1	1,8-3,8	1,4	0,98-2,7

[1]IQR: Amplitud interquartílica (P25-P75); * *p-valor*<0,05 pel test Mann-Whitney

Durant la barreja els treballadors participants a l'estudi utilitzen guants, semimàscara i gorra o barret (Figura 4.20). En general, aquells que mai no els utilitzen tenen concentracions més altes que aquells que els utilitzen sempre o a

vegades. En el cas dels guants, aquestes diferències són significatives ($p<0,05$; test U de Mann Whitney), entre els que els utilitzen sempre i a vegades pel metabòlit TCPY i els que els utilitzen a vegades i mai pel PNP i el TCPY. En el cas de màscara, només s'observa una diferència significativa pel metabòlit PNP entre els que l'utilitzen a vegades o mai.

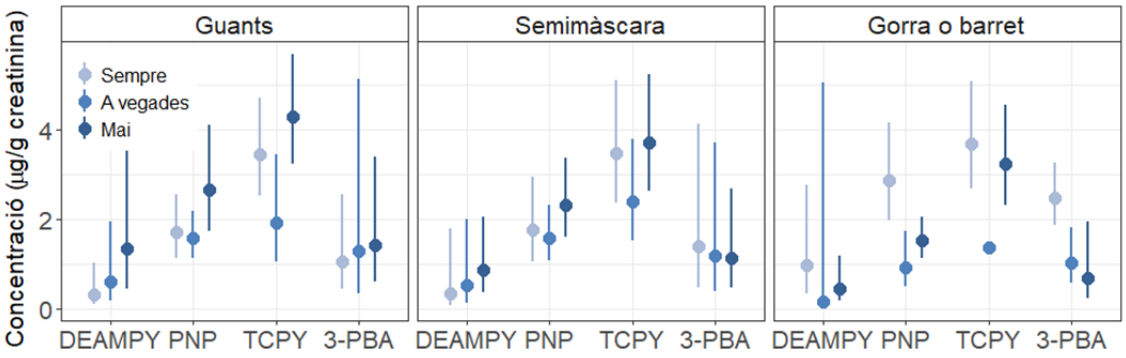

Figura 4.20 Mitjana geomètrica de la concentració dels diferents metabòlits segons els diferents EPI utilitzats durant la barreja.

En quant l'ús de la gorra o el barret la tendència general és totalment contrària. Aquells que l'utilitzen sempre tenen concentracions més elevades que aquells que l'utilitzen a vegades o mai. Sent aquestes diferències significatives pel PNP, TCPY i 3-PBA. A diferència dels guants i la semimàscara, que s'utilitzen per protegir la pell i el sistema respiratori, respectivament, la gorra o el barret són per protegir-se del Sol. Si aquesta peça de roba no es renta bé després de la manipulació dels productes pot arribar a ser una font d'exposició contínua.

Durant l'aplicació els participants només feien servir semimàscara i gorra o barret (Figura 4.21). Com s'ha vist abans més de la meitat dels treballadors fan servir tractor amb cabina per fumigar els camps, per tant durant l'aplicació no estan tan exposats com durant la barreja. La majoria d'ells mai fa servir màscara (80%), aquestes diferències fan que els 3 grups estudiats no siguin homogenis obtenint resultats amb molta variabilitat. En el cas d'utilització de gorra o barret la tendència és la mateixa que en el moment de la barreja, sent les diferències estadísticament significatives ($p<0,05$) pel metabòlit PNP.

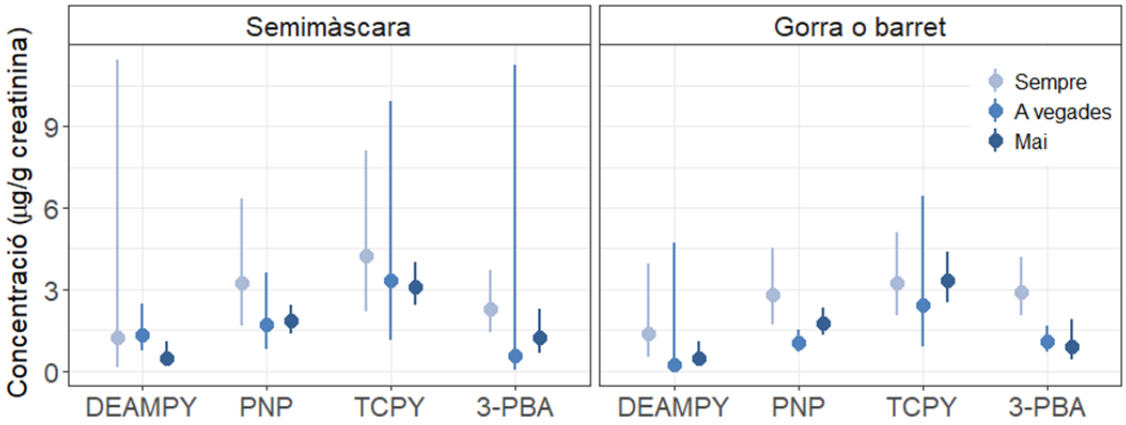

Figura 4.21 Mitjana geomètrica de la concentració dels diferents metabòlits segons els diferents EPI utilitzats durant l'aplicació.

Variabilitat dels metabòlits de pesticides OP i PYR en orina

Els pesticides OPs i els PYR tenen una vida mitjana molt curta dins el cos humà (Needham, 2005). Alguns estudis suggereixen que una mostra única d'orina pot donar informació de l'exposició a una font coneguda (Fenske et al., 2000). Per altra banda, altres estudis han avaluat la variabilitat de les concentracions dels pesticides OPs i PYR en diferents períodes de temps (Bradman et al., 2013; Spaan et al., 2015; Cequier et al., 2017; Hioki et al., 2019), concloent que existeix una variabilitat en l'exposició de pesticides OPs i PYR en una persona però que es pot disminuir dissenyant correctament els estudis. Per exemple, prenent la mostra durant la tarda i analitzant les dades per quartils (Hioki et al., 2019) o allargar els temps de recollida de mostra durant un mínim de 48 h (Egeghy i Lorber, 2011).

A l'ARTICLE 7 s'ha avaluat la variabilitat durant l'embaràs de l'exposició a pesticides OP en dones, prenent mostres d'orina del primer, segon i tercer trimestres. Per tal d'avaluar aquesta variabilitat, s'ha calculat el coeficient de correlació intraclasse (ICC) per tots els metabòlits detectats en més del 35% de les mostres (DEAMPY, IMPY i PNP). L'ICC és un estadístic descriptiu que

s'utilitza normalment per quantificar la fiabilitat de les mesures associades a variables quantitatives continues. La fiabilitat dels resultats es caracteritza com a pobra (ICC < 0,40), suficient (0,40 ≤ ICC < 0,60, bona (0,60 ≤ ICC < 0,75) o excel·lent (≥ 0,75) (Rosner, 2011).

D'acord amb els coeficients observats, la reproductibilitat dels pesticides OP al llarg dels tres trimestres és pobra pel DEAMPY i suficient pel PNP i l'IMPY (resultats ajustats). Aquests ICCs són consistents amb els descrits en un estudi avaluant el metabòlit PNP durant l'embaràs en dones de Puerto Rico (Lewis et al., 2015) on van trobar que els ICCs eren de 0,31 i 0,28 pels nivells sense corregir i corregits, respectivament. En una comparació de la variabilitat diürna, altres estudis han trobat ICCs suficients per a tots els compostos (Egeghy i Lorber, 2011; Cequier et al., 2017). En quant períodes més llargs de temps, per exemple 18 setmanes (Spaan et al., 2015) o un any (Attfield et al., 2014), els ICCs van de 0,08 a 0,38. Per tant, una valoració de l'exposició a pesticides OP al llarg del temps requereix de l'anàlisi de més mostres (Casas et al., 2018).

4.5 Conclusions

En aquest capítol s'ha presentat un nou mètode per l'anàlisi de pesticides OP i PYR i s'ha utilitzat per conèixer les concentracions en diferents tipus de poblacions, exposades i no exposades. S'han examinat alguns dels determinants que influeixen a aquesta exposició i s'ha avaluat la variabilitat entre trimestres d'una població de dones embarassades. A continuació es resumeixen les conclusions més importants extretes dels estudis d'aquest capítol.

Desenvolupament d'una nova metodologia analítica per a l'anàlisi de pesticides OP i PYR. El mètode desenvolupat en aquest treball es basa en l'extracció en fase solida i posterior anàlisis per UPLC-MS/MS. Aquest és adequat per l'anàlisi de metabòlits de pesticides organofosforats i piretroides en mostres d'orina d'adults i nens exposats i no exposats. Permet la determinació

de 8 compostos diana amb sensibilitat, exactitud i precisió satisfactòries. Per contra, aquesta metodologia no és vàlida per la determinació de piretroides en dones embarassades. Aquest tema requereix de més estudi per poder determinar les causes per les quals els piretroides no són analitzables en aquesta matriu.

Detecció i nivells de pesticides OP i PYR a Itàlia, Eslovènia i Espanya. En tots els participants dels estudis realitzats es mostren nivells detectables tant de pesticides OP com de PYR. Això indica que tot tipus de poblacions estan exposades, fins i tot les que viuen lluny d'una font d'emissió. En general, la població més exposada és la formada per treballadors agrícoles i població rural, com Sucs. Per la resta de poblacions, exceptuant el DEAMPY, les concentracions de metabòlits són equivalents a les descrites per a altres poblacions d'arreu del món. El metabòlit del pesticida pirimifos presenta nivells anormalment alts a les poblacions de Trieste i Tarragona, aquest fet suggereix una exposició puntual en aquestes ciutats o l'ús extensiu, ja que el pirimifos és un pesticida utilitzat per l'emmagatzematge de cereals, un grup d'aliments molt produït i consumit en regions mediterrànies.

Comparació entre les mares i els seus fills de Ljubljana. A l'estudi de Ljubljana es van comparar els nivells de metabòlits a mares i els seus fills. Considerant que l'exposició a través de la dieta i l'estil de vida és similar, els nens presentaven concentracions significativament més elevades de DEAMPY, IMPY i 3-PBA que les seves mares, per contra el TCPY era significativament superior a les mares. Tot i que la raó d'aquests resultats necessita més investigació, s'ha suggerit que la diferència pot ser deguda a una major ingesta per un pes més baix en el cas dels nens o que aquests tinguin un metabolisme oxidatiu més efectiu a l'hora d'eliminar-los.

Estimació del consum diari de pesticides OP i PYR. Als estudis d'Itàlia i Eslovènia es va estimar el consum diari (EDI) de pesticides a través de la dieta a partir de les dades de biomonitorització i comparació amb el consum diari

acceptable (ADI) publicats per a cada compost. En tots els casos tots els EDI estaven per sota dels ADI, dins dels nivells segurs. No obstant, és necessària una estimació fiable del factor d'excreció urinària tant en nens com en adults per avaluar correctament els EDI.

Influència de factors sociodemogràfics en l'exposició a pesticides OP i PYR. L'educació familiar es va relacionar amb la concentració en orina del metabòlit DEAMPY en els nens de Trieste, una educació superior significava una exposició major de pirimifos. A la resta d'estudis no es va trobar aquesta diferència. Els índex de massa corporal normal es va relacionar amb nivells més baixos del metabòlit TCPY en les mares de Ljubljana, però aquesta diferència no s'ha observat en la resta d'estudis. Tant la influència de l'educació com del IMC s'han atribuït a tenir estils de vida més saludables, seguint una dieta, probablement, amb un contingut superior de fruites i verdures. El factor d'exposició més important que s'ha determinat és la residència. S'ha vist que viure en un entorn rural o treballar al camp representa una exposició major a pesticides OP i PYR.

Avaluació de l'exposició d'una població agrícola. S'ha avaluat l'exposició a pesticides OP i PYR i la seva relació amb la manipulació i utilització d'equips de protecció individuals en la població agrícola de Sucs. Treballar amb un tractor amb cabina disminueix significativament els nivells de metabòlit DEAMPY en l'orina dels treballadors. Sobre els EPI que s'utilitzen durant la barreja i l'aplicació, s'ha trobat mentre que l'ús de guants i semimàscara, en general, protegeix de l'exposició, però no s'ha observat una relació tan directa entre l'ús de la semimàscara durant l'aplicació. Per contra, tant durant la barreja com l'aplicació l'ús continuat de gorra o barret fa que els treballadors estiguin més exposats, fet atribuïble a una poca higiene vers aquesta peça de roba, que si no es renta després de cada utilització pot arribar a ser una font d'exposició contínua.

Variabilitat dels pesticides OP i PYR en orina. Ambl el càlcul dels coeficients de correlació intraclasse es va determinar que la variabilitat entre els trismestres de l'embaràs d'una població de dones de Tarragona era entre pobra i suficient, tot concloent que una valoració a l'exposició de pesticides OPs al llarg del temps requereix de l'anàlisi de diverses mostres.

CAPÍTOL 5. CONCLUSIONS

Capítol 5: Conclusions

5.1 Consideracions finals

Un dels objectius en general a l'hora de mesurar l'exposoma recau en identificar i caracteritzar les exposicions ambientals de manera directa i amb la major precisió possible. Aquesta tasca és imprescindible per conèixer la relació existent entre els contaminants ambientals i la salut humana. Aquest estudi s'ha nodrit de la natura multidisciplinària de l'epidemiologia ambiental. Per fer possible aquest treball han col·laborat investigadors provinents de diferents disciplines, metges, químics, ambientòlegs o epidemiòlegs.

La rellevància i credibilitat dels resultats d'aquests tipus d'estudis depenen dels mètodes analítics i tècnics. En primer lloc és essencial obtenir una mesura robusta dels nivells d'exposició, especialment en termes d'exactitud i fiabilitat, altrament es poden obtenir resultats esbiaixats o subestimats. A més, es requereix d'una mida de mostra relativament gran, representant població general o altres tipus de poblacions acompanyades d'informació detallada sobre els individus avaluats, com per exemple aspectes físics (com edat, sexe o índex de massa corporal), característiques sociodemogràfiques (com nivell educacional, estatus social o tipus d'ocupació) i factors ambientals (proximitat a un focus d'exposició). A continuació, s'avaluen acuradament els resultats i paràmetres, es verifiquen estadísticament i es contrasten amb els publicats en la literatura. Finalment, es plantegen hipòtesis que s'analitzen, i o s'adopten o es

rebutgen. Es formulenalgunes conclusions i recomanacions així com noves preguntes per ser respostes en investigacions futures.

En els estudis realitzats en el context d'aquesta tesi s'ha seguit aquesta estratègia de treball. Així doncs s'ha desenvolupat una metodologia analítica per detectar de manera fiable pesticides organofosforats i piretroides. S'ha estudiat el grau d'exposició de diferents poblacions a compostos organoclorats, organobromats i organofosforats, tot avaluant simultàniament la implicació de diferents factors individuals, sociodemogràfics i mediambientals. Per tant, en aquesta tesi ss'ha realitzat treball analític, anàlisis estadístics, identificació de tendències i patrons, comparació amb la literatura existent i discussió d'hipòtesis diverses.

5.2 Conclusions

Aquesta memòria de tesi doctoral ha contribuït a ampliar la caracterització de l'exposoma de diferents poblacions. S'ha incrementat la informació disponible sobre l'exposició a contaminants orgànics persistents, pesticides organofosforats i piretroides en diferents poblacions humanes i sobre alguns dels factors que la poden determinar. També, inclou un dels primers estudis realitzats en una cohort amb un número de mostres rellevant de l'hemisferi sud.

Atès que les conclusions específiques de cada capítol ja s'han presentat, a continuació només s'indiquen els resultats més rellevants.

Acumulació dels COPs en poblacions remotes. Argentina i Chukotka són indrets considerats remots en quant a contaminació de compostos organoclorats i organobromats persistents, perquè aquests no s'han produït ni utilitzat, en gran quantitats, en aquestes zones. Tot i això totes les dones participants en els estudis tenen nivells detectables d'algun dels compostos analitzats. Aquest demostra l'impacte d'aquests compostos i persistència en el

medi ambient fins i tot anys després d'haver estat prohibits o restringits. Aquest treball confirma que les concentracions de compostos organoclorats augmenten amb l'edat i disminueixen amb la paritat. S'ha vist que el lloc de residència, i fins i tot viure sempre en el mateix lloc o marxar temporades, té un paper molt important en l'exposició a COPs, per exemple viure en zones rurals o a la costa representa un risc major d'acumulació d'alguns compostos organoclorats.

Efectes dels OCs sobre nounats. S'ha observat que les mares de Chukotka que tenien concentracions altes de 4,4'-DDT tenien edats gestacionals més llargues i nadons amb pes i longitud més gran.

Efectes de la destil·lació global. Amb l'estudi d'Argentina s'ha pogut observar que a l'indret més fred (Ushuaia) les concentracions dels compostos més volàtils eren més altes i a l'indret més calorós (Salta) la tendència era la contraria.

Desenvolupament d'una nova metodologia analítica per l'anàlisi de pesticides OPs i PYR. El mètode desenvolupat ha permès l'anàlisi de diferents famílies de pesticides (OPs i PYR) en orina en una gran quantitat de mostres de manera ràpida, robusta i econòmica, tot fent servir petits volums de mostra. Aquest mètode és adient per l'anàlisi d'orina de tot tipus de poblacions excepte dones embarassades on només es detecten els pesticides organofosforats.

Nivells i patrons d'exposició d'OPs i PYR. En població general, no exposada directament, factors associats a una vida saludable (educació familiar alta o IMC saludable) s'han relacionat amb nivells més alts de pesticides OPs i PYR, tot atribuint aquests fets a dietes amb un contingut més alt de fruites i verdures. De la mateixa manera que els COPs, la residència és un determinant molt important de l'exposició a pesticides, viure en un entorn rural o treballar al camp representen una exposició més alta. En poblacions laboralment exposades és molt important la gestió que fan dels equips de protecció individual, perquè no fer-los servir o fer-los servir malament representen un risc pels treballadors.

CAPÍTOL 6. BIBLIOGRAFIA

Capítol 6: Bibliografia

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